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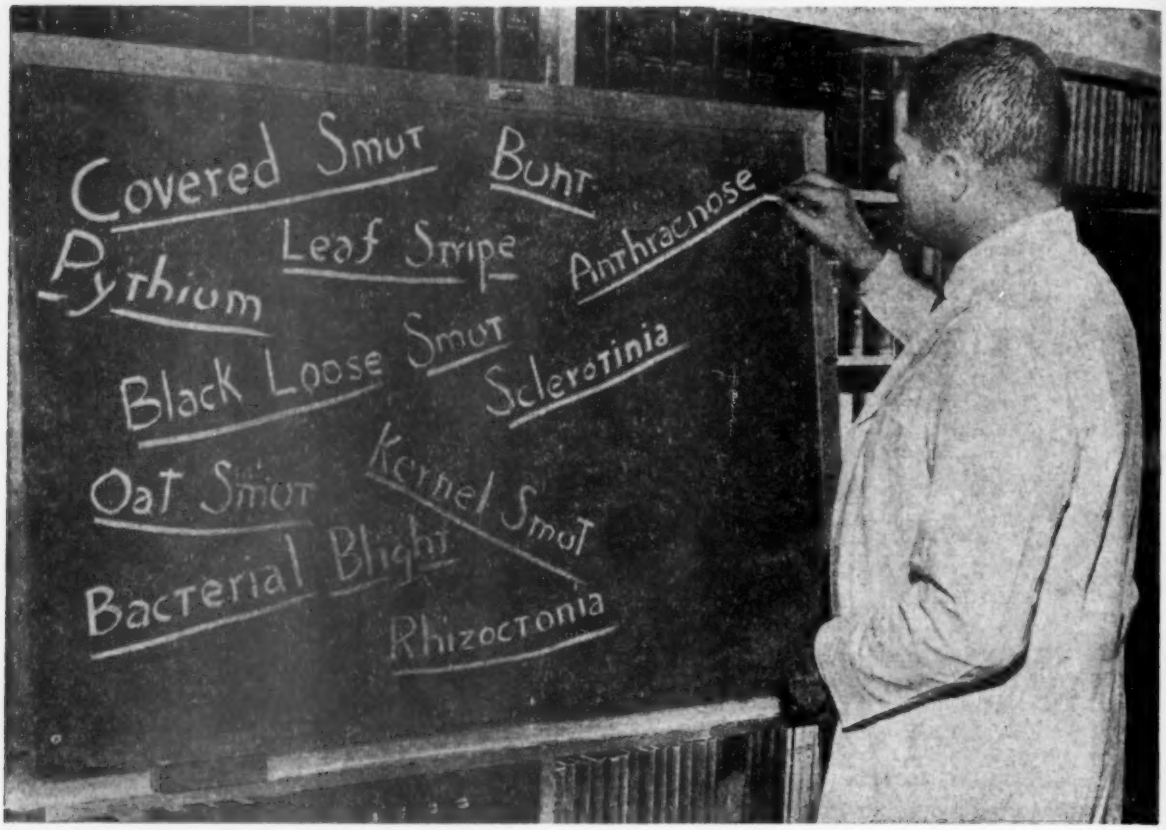

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THE PERSISTENCE OF NONVOLATILE DIFFUSIBLE FUNGICIDES IN SOIL[1]

Donald E. Munnecke

SUMMARY

Twenty ml of 1000 ppm (active ingredient) of solutions or suspensions of 4 nonvolatile, diffusible fungicides were added to flasks containing 30 g of a mixture (sterilized with steam or propylene oxide, or untreated) of peat moss and fine sand (1:1 by weight) plus a full complement of fertilizers. Samples of the soil were bioassayed for diffusible fungicidal activity at intervals up to 150 days after the fungicides were applied. Under these conditions, captan was very stable, Semesan was rapidly inactivated biologically, and nabam and ferbam were inactivated nonbiologically. Semesan also declined nonbiologically, a slow-acting factor being operative in the absence of microorganisms. A very rapid loss of diffusible activity, which occurred within 10 hours after nabam was applied to soil, was primarily a chemical reaction, but multiple factors were involved.

Fungicide soil drenches are commonly used for control of soil-borne plant pathogens. Most applications are derived from empirical procedures. Relatively little is known of the fate of the nonvolatile fungicides after they are applied to the soil. This paper describes the behavior of 4 nonvolatile fungicides in aqueous suspensions or solutions applied to soil in the laboratory, and discusses some of the factors that affect their persistence.

Richardson (15) is one of the few who have studied the persistence of fungicides in soil and related it to soil microbial activity. He showed that thiram persisted over 2 months in sandy soil but disappeared within 1 week in compost soil. His curve representing the decline of thiram in soil with time suggested that more than 1 process was involved in its disappearance, and that microorganisms were one of the factors responsible for its breakdown. In thiram-treated soils Trichoderma, Penicillium, and bacteria increased; Actinomycetes decreased in a sandy soil, but not in compost soils.

Rushdi and Jeffers (16) studied fungicide persistence in soils in tubes by measuring their effect on the growth of *Rhizoctonia solani* Kuhn. They concluded that soil texture, pH, and organic matter, probably since they affect the absorptive capacity of soil, are the most important factors affecting the activity of soil fungicides. Unfortunately, they studied only autoclaved soils, so the biologic factors could not be evaluated.

In contrast to the relative lack of information on the fate of fungicides in soil, there is considerable literature on the breakdown of antibiotics and herbicides.

Gottlieb and co-workers, in a series of papers on the production and role of antibiotics in soil (3, 4, 11, 12, 18), showed that both physicochemical and biological factors were operative. The adsorptive capacity of colloidal complexes, such as clays and organic matter, varied with the type of antibiotic molecule, i.e. basic antibiotics were strongly bound, neutral antibiotics were weakly bound, and the acidic compounds were intermediate in their reactions. Wright (20) found that the persistence of gliotoxin in soil depended most on an adequate food supply for the producing organism, *Trichoderma viride* Pers. ex Fries, and on a lowered pH, whereas griseofulvin, produced by *Penicillium nigricans* Bainer, in addition to depending on a high nutrient level, was also influenced by other microorganisms. She further reported (20) that *Mucor rammannianus* Moeller slowly decomposed griseofulvin, and that other, unnamed organisms were capable of decomposing it rapidly. Pramer and Starkey (14) showed that streptomycin was completely inactivated in nonsterile soil in 2 weeks but was still active in sterile soil after 3 weeks. A *Pseudomonas* sp., isolated from streptomycin-treated soil, was found capable of inactivating the antibiotic *in vitro*.

As with the antibiotics, there is considerable variation in the behavior of herbicides in soil. Bell (2) cited 13 papers in which it was shown that 2,4-dichlorophenoxyacetic acid was inactivated in soil by bacteria. Jensen (7) described a group of Pseudomonas-like bacteria, a nonspore-forming bacterium, and an Agrobacterium group that were capable of decomposing chloro-substituted aliphatic acids, and cited other authors who have shown that microorganisms play an active role in the breakdown of soil herbicides.

The influence of nonbiologic factors in the breakdown of herbicides has been studied. Harvey (5) found that 2,4-D disintegrated faster in acid soils than in neutral or basic soils. The herbicide was fixed in clay soils and could not be leached out by flushing with water. Levi and Crafts (8) found that maleic hydrazide was similar to 2,4-D in being strongly adsorbed by clays with a high anion exchange capacity, but differed in that it could be leached out. The rate of decomposition increased in warm moist soils. Another compound, pentachlorophenol, was found by Harvey and Crafts (6) to be stable for 12 months in soil, not fixed in clay, and leachable from soil.

Thus, it is known that the persistence of fungicides, antibiotics, and herbicides in soils varies with the type of compound, the activity of soil microorganisms, and physicochemical factors of the soil, such as tex-

[1] Accepted for publication June 3, 1958.

ture, pH, ion exchange capacity, organic matter content, moisture, and temperature. In the work reported here the effect of physicochemical factors and biological factors on the persistence of 4 fungicides was studied.

MATERIALS AND METHODS.—The fungicides were studied by adding aqueous solutions or suspensions of them to autoclaved, propylene-oxide-treated, or untreated soil in cotton-stoppered flasks. Periodically, samples of the soils were biologically assayed for fungicide activity. Activity curves for the fungicides in sterile and nonsterile soil were compared to determine the factors involved in their breakdown.

Soil used.—A UC-type soil mix (1) containing by volume 50% fine sand and 50% German peat moss was used. Fertilizers (potassium nitrate, 10 g; potassium sulfate, 10 g; single super phosphate, 100 g; dolomite lime, 280 g; oyster shell lime, 100 g) were mixed well in 2 bushels of soil and dried to constant weight at temperatures below 40°C. The pH was 5.7. Erlenmeyer flasks (125-ml) each received 30 g of dried soil and were plugged with cotton.

Soil treatment.—The flasks were divided into 3 lots: one sterilized 1 hour at 10–12 lb. p.s.i. steam pressure, one sterilized with gaseous propylene oxide, and one untreated. For propylene-oxide sterilization, plugs and the unplugged flasks were placed in a steel container with a dish containing 1 ml of propylene oxide per liter of space. The container was sealed 24 hours with 2 layers of polyethylene. The flasks were then aerated 24 hours, replugged, treated again while plugged, and stored until used.

The effectiveness of sterilization was verified by plating samples of the soil, using the dilution plate technique. No growth occurred from soil treated with steam or propylene oxide, whereas about 1 million fungal colonies and 2 3 million bacterial colonies were produced per g of untreated soil.

Fungicide application.—Concentrations of 1000 ppm (active ingredient) of solutions or suspensions of the following fungicides were used: Captan 50-W (50% captan, *N*-(trichloromethylthio)-4-cyclohexane-1,2-dicarboximide); Dithane D-14 (19% nabam, disodium ethylenbis(dithiocarbamate)); Semesan (30% 2-chloro-4-(hydroxymercuri)phenol); Fermate (76% ferbam, ferric dimethyldithiocarbamate).

The fungicides were chosen because they are used in practice as soil drenches and because each is distinctly different from the other. Nabam contains no heavy metal and is soluble in water, and considerable information has been accumulated on its breakdown products (9, 10, 17). Ferbam is similar to nabam in that it is a carbamate compound, but it is insoluble and contains iron, in addition to other differences in molecular structure. Captan is an entirely different type of organic compound, and Semesan is an organic mercurial compound.

All of these chemicals reacted differently in soil. Captan was very stable, nabam and ferbam were inactivated nonbiologically, and Semesan was rapidly inactivated biologically. It should be emphasized that these results may pertain only to this 1 soil mix and pH.

Twenty ml of one of the fungicides were added to each flask, as aseptically as possible, and the flasks replugged. After thoroughly shaking to wet the soil, the flasks were stored in the dark at 65–70°F until assayed. The flasks were individually weighed, and the amount of evaporation was occasionally measured, by reweighing them. Water loss during the first 6 weeks was not significant. After 2 months, which usually ended an experiment, the water loss was 7%. Before assaying these older samples, sufficient sterile distilled water was added to restore them to their original weights.

Fungicide assay.—The fungicidal activity in soils was determined by loosely packing soil in plastic rings, extruding the plug on to the surface of an agar plate previously seeded with spores of *Myrothecium verrucaria* (Alb. & Schwein.) Ditm. ex Fr., and measuring the clear zone remaining around the plug. The technique has been reported in detail by Munnecke (13).

The soils were initially assayed within 2–4 hours after the fungicides were applied, and thereafter at intervals of 1, 2, 4, 7, 14, 21, 30, and 63 days. Two later experiments were similar, except that, in the last one, additional assays were made ½, 2, and 4 hours, and 150 days after fungicide application. Three flasks were used for each fungicide and soil treatment each time. Two soil plugs from each flask were assayed, giving 6 assays for each sample each time. After 48 hours the diameter of the clear zone remaining around the soil plugs was recorded. The data were evaluated by plotting the diameters of clear zones against the time after fungicide application.

Quantitative estimates of fungicide activity were not attempted. Standard dosage-response curves were plotted for each period by assaying samples of steamed soil to which a graded series of doses of 1 or more of the fungicides had been applied 1½ hours previously. By comparing the slopes and positions of the curves with those obtained from other experiments it was possible to determine whether apparent increases or decreases in fungicide activity were due to the fungicide or to variations arising at different times of assay. In addition, the dosage-response curve made with each assay could be used to correct the data of a single assay to allow for uncontrolled variations between assay periods.

RESULTS.—*Persistence of nabam.*—The most striking response of nabam was a rapid loss of activity in soil. In the first 3 experiments the assays were made about 1½ hours after fungicide application and at varying periods thereafter. The data (Fig. 1) indicate that the fungicide underwent rapid changes in the first few hours. Later experiments included assays immediately after fungicide application and after ½, 1, 1½, 2, 4, 8, 10, and 22 hours (Fig. 2). It is appar-

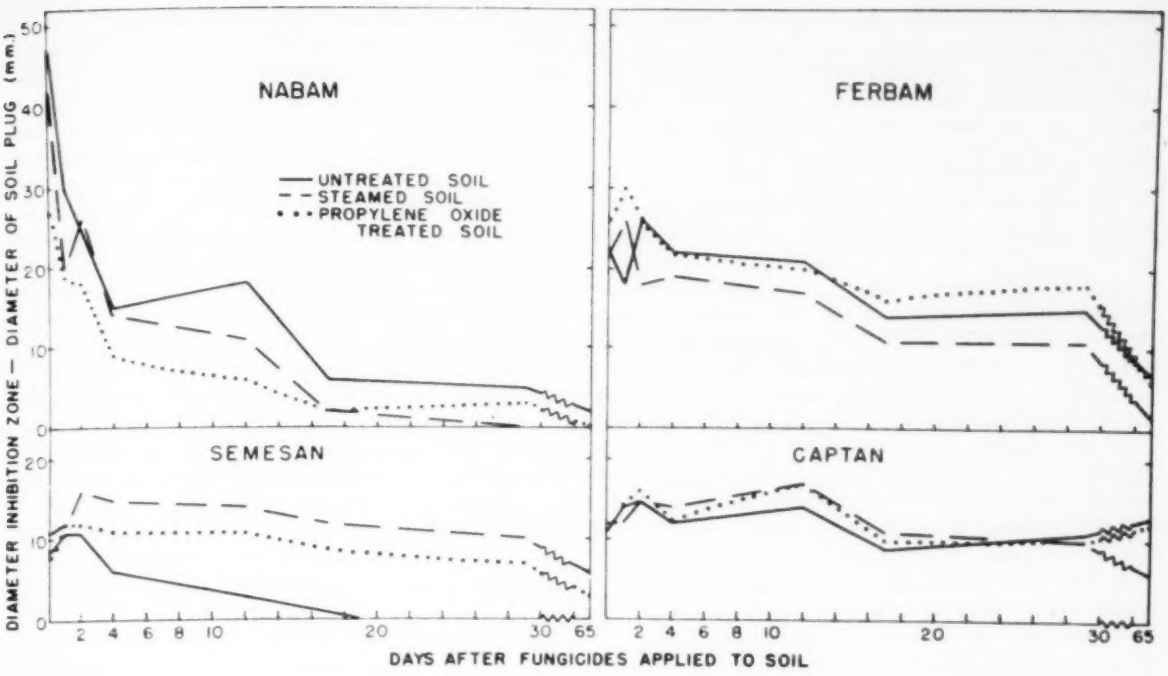

FIG. 1. Persistence of 4 fungicides in soil.

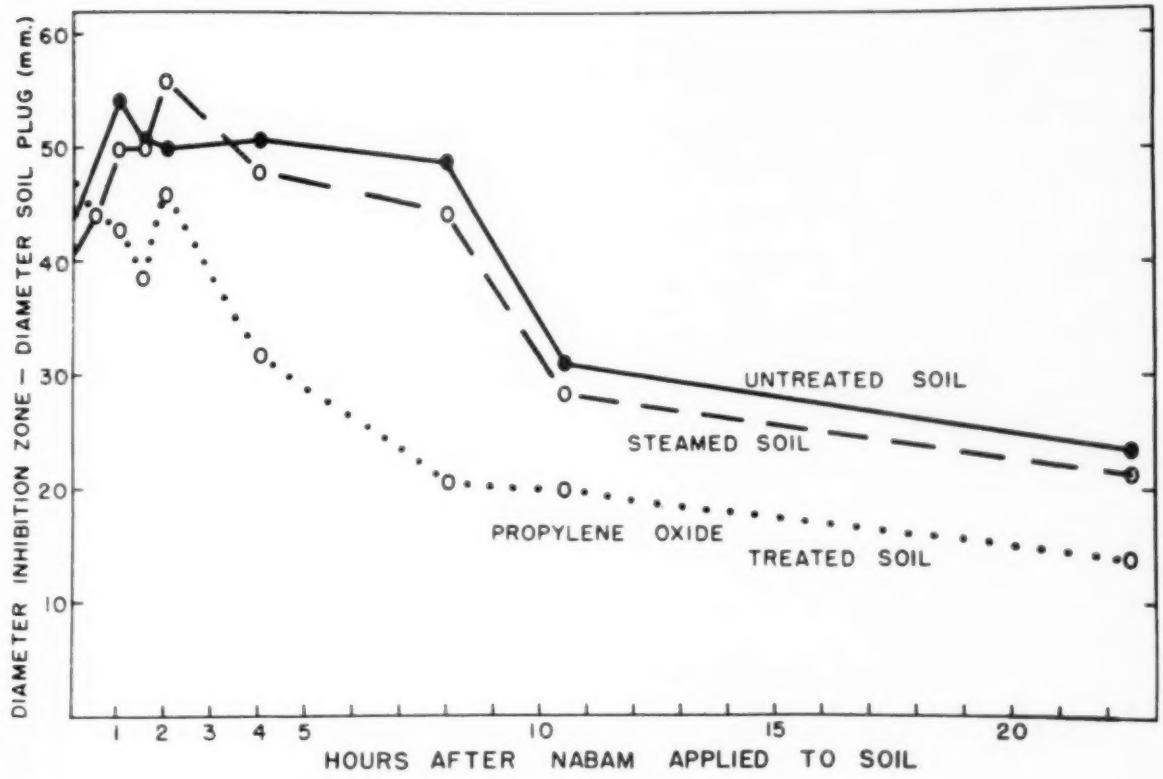
UNTREATED SOIL
STEAMED SOIL
PROPYLENE OXIDE TREATED SOIL
DIAMETER INHIBITION ZONE — DIAMETER SOIL PLUG (mm.)
HOURS AFTER NABAM APPLIED TO SOIL

FIG. 2. Loss of nabam activity in the first 24 hours after application to soil treated as indicated.

ent not only that nabam activity decayed rapidly but that the decay was nonbiologic, since the curves in sterile soil were essentially the same as those in nonsterile soil. One striking result was the more rapid decay in propylene-oxide-sterilized soil than in steamed and unsterilized soils.

The pH of the soils conceivably could account for the faster decay in propylene-oxide-treated soils, for the stability of nabam solutions varies with changes in pH (9, 19). The initial pH of soil treated with propylene oxide was 6.4, whereas the pH of untreated soil was 5.5. The addition of the alkaline nabam solution raised the pH of the propylene-oxide-treated soil to 6.6, and that of unsterilized soil to 5.9. The pH rose in steamed and untreated soils until the seventh day, but decreased slightly in the propylene-oxide-treated soils. In 31 days the pH of all 3 soils was the same. From the above considerations it is oversimplification to assume that the decrease in the diffusible activity of nabam in propylene-oxide-treated soil is due solely to pH effects. It is probable, however, that the initial differences in pH of the 3 soil treatments is allied in some way to the early differences in fungicide activity.

To separate physical factors in the soil from chemical factors, a 1000-ppm solution of nabam was placed in a dark, cotton-stoppered bottle and the solution bioassayed at intervals by the filter paper disk technique (Fig. 3). The fungicidal activity decreased in much the same fashion as in soil, except that it was stable for 2 days before it rapidly declined. Odor, pH, and color changed very definitely on standing. These data support the postulation that the decline of nabam in soil was primarily due to chemical reactions, but that the processes were speeded in soil.

Persistence of ferbam.—The curves of the persistence of ferbam (Fig. 1) were similar to those obtained for nabam except that they were less steep, and the reactions were similar in all soil treatments. The diffusible constituent of ferbam was obviously more stable than that of nabam. Further, the decay was due to physicochemical factors and not biological ones, since the reactions were essentially similar in the presence or absence of organisms.

Persistence of Semesan.—The outstanding result with Semesan was the marked inactivation of the fungicide in nonsterile soil. The curves of Fig. 1 indicate that several factors operated in the decline of Semesan. It is obvious that organisms were capable of quickly breaking down the fungicide. The curves of the steamed and propylene-oxide-treated soils show the presence of a nonbiologic factor, and their linear aspect suggests that a single factor was operative in the absence of organisms. In 1 of the 3 experiments used to compile Fig. 1, Semesan in the untreated soil was inactivated so rapidly that all trace of the diffusible constituent of the compound was gone in 10 days. The rate of decay was not so rapid in other experiments: 18 days on the average.

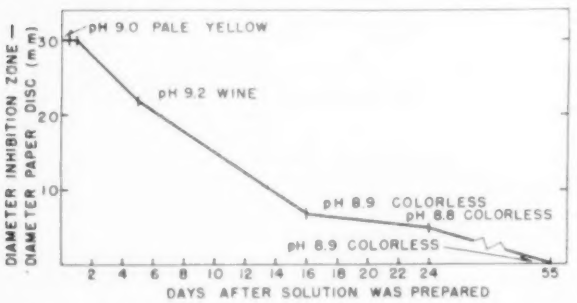
DIAMETER INHIBITION ZONE − DIAMETER PAPER DISC (m m)
30
20
10
pH 9.0 PALE YELLOW
pH 9.2 WINE
pH 8.9 COLORLESS
pH 8.8 COLORLESS
pH 8.9 COLORLESS
2 4 6 8 10 12 14 16 18 20 22 24 55
DAYS AFTER SOLUTION WAS PREPARED

FIG. 3. Loss of nabam activity in aqueous solution in the absence of soil.

The parallelism of the curves of the steamed and propylene-oxide-treated soils is difficult to explain. Treatment with propylene oxide raised pH ½–1 unit, which may have influenced these reactions. Also, steamed soils were more easily wetted than the other 2, so, possibly, less Semesan was sorbed by the steamed than by the propylene-oxide-treated soils. In any case, the differences were consistent and the greater activity occurred in the steamed soils.

The organisms responsible for the decline of Semesan are subjects for future experimentation. Experiments showed that soil fungi increased moderately in Semesan-treated nonsterile soil for the first few weeks, reaching a maximum about when the diffusible activity of Semesan was at a minimum. Fungi isolated from Semesan-treated soils were highly tolerant of Semesan incorporated in agar media. Early results indicated that some of the soil fungi (*Penicillium* and *Aspergillus* spp.) were able to adapt readily to Semesan in agar.

Persistence of captan.—Captan was remarkable in that diffusible activity after 65 days nearly equalled that initially present (Fig. 1). The soil treatments had no effect on persistence. With the soil and conditions of these experiments, captan was extremely stable.

DISCUSSION.—The loss of activity of the carbamate fungicides, nabam and ferbam, was nonbiologic. Ferbam was more persistent in the soil than nabam, and the changes in diffusible activity were less abrupt. The slopes of the curves for the breakdown of nabam are very similar to those that Richardson (15) reported for the breakdown of thiram in soil. As he noted for thiram, the curves, being neither linear nor logarithmic, indicate that multiple factors are operative in the decay of nabam.

It is possible that in a soil containing greater numbers of organisms, or different types, more biological decay might result. As one of the reviewers of this paper noted, the amounts of fungicides applied may have been sufficient to lower the inoculum in unsterilized soil to a point where it would be impossible to measure biologic inactivation. Although this is possible, it is not likely, since there were sufficient organisms present to decay Semesan rapidly, and soil platings of treated soils showed that considerable numbers of organisms were present, though exact

figures were not kept.

The fact that fungicides vary in their ability to persist and to penetrate soils is an important consideration in the use of nonvolatile chemicals as soil drenches for plant disease control. It may be desirable that fungicides decay in soil, since phytotoxic amounts of the chemical may accumulate in the soil. This could especially be the case with molecules containing mercury, copper, or manganese, for example. Conversely, the chemicals should persist long enough to be fungicidal to soil organisms and to obviate the need for numerous applications to the soil to achieve successful control.

The rapid loss of diffusible activity that occurred within the first few hours after nabam was applied to soil is a factor that must be considered in studies of persistence of fungicides of this nature. If assays are delayed too long after such a fungicide is applied a misleading value may be obtained from the assay.

Another important consideration regarding the control of soil-borne pathogens is that chemicals may control root disease fungi indirectly by altering the biologic environment. The use of carbon disulfide for control of Armillaria root rot is an example. By studying the persistence of fungicides in soil it may be possible to develop chemicals that are of low direct fungitoxicity but of long persistence in soil, so that a continued favorable balance of microorganisms may be maintained.

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NEMATODE VECTOR OF SOIL-BORNE FANLEAF VIRUS OF GRAPEVINES[1]

Wm. B. Hewitt, D. J. Raski, and A. C. Goheen[2]

SUMMARY

Experiments in the vineyard and in containers under controlled conditions demonstrate that fanleaf disease of grapevines in California is soil-borne and not air-borne, and that it is transmitted by *Xiphinema index* Thorne & Allen.

Screenings containing nematodes from fanleaf soil, passed through a 40-mesh screen and collected on a 200-mesh screen, transmitted fanleaf virus to healthy *Vitis vinifera* L. var. Mission growing in clay pots. *X. index* hand-picked from such screenings transmitted fanleaf virus to healthy Mission and to *V. rupestris* Scheele var. St. George growing in clay pots. *X. index* from the root zone of healthy Tokay grapes and from the root zone of figs, in separate tests, transmitted fanleaf from fanleaf-diseased St. George to healthy St. George growing in the same clay pot. In each test, healthy St. George growing with fanleaf St. George in clay pots remained healthy in the absence of nematodes.

Fanleaf is a destructive virus disease of grapevines in California vineyards (20). Leaf deformity and mottling, marked reduction in crop, and vineyard decline are characteristic effects of the disease on several of the grape varieties important in the California grape industry. The disease is widespread in the state, and in some localities its incidence is high (9).

This paper reports experiments on the method of spread of this virus in California, and is believed to be the first report of transmission of a plant virus by a nematode vector.

Grapevine diseases similar to fanleaf, and apparently identical (19, 20), are known by different names (gabler, roncet, arricciamento, court-noué, Reisigkrankheit, urticado, etc.) in different countries. The term "infectious degeneration" applies to a disease complex (30); "Milink" mosaic in Czechoslovakia (47) is apparently caused by a different virus, but may be a part of the complex. The rather extensive literature on these diseases has been generally reviewed, and at intervals reviewed for specific purposes (1, 2, 5, 10, 11, 13, 16, 17, 22, 27, 29, 32, 33, 35, 40, 49, 50, 51, 52).

It has been generally known for many years that normal grapevines will become diseased in a relatively short time if planted where old vineyards have been removed because of vineyard degeneration. An early account is by Rathay (39), published in 1882. In 1918 Petri (33) presented experimental evidence of the soil-borne nature of arricciamento (also called roncet and court-noué). Later work of Petri (34, 35) and Ravaz (40, 41) established that grapevines can contract the disease from infested soil. Evidence of the soil-borne nature of the disease has also been presented by many others (6, 7, 8, 11, 12, 14, 18, 21, 23, 24, 25, 26, 28, 36, 42, 52).

In 1937 Branas, Bernon, and Levadoux (7) presented a hypothesis, based mainly on observation, that Phylloxera were most likely responsible for the spread of court-noué. In 1946 (8) they presented experimental evidence in support of their observations, but did not prove that Phylloxera transmit the disease. On the other hand, Lykiardopoulo (25) observed the spread in Greece of court-noué in ungrafted vines of *Vitis vinifera* in localities without Phylloxera. Nysterakis (28) demonstrated that Phylloxera are not natural vectors of the virus that causes court-noué. Vuittenez (52) also was unable to show that Phylloxera are vectors of the court-noué virus.

Ravaz (40) reported very good control of court-noué from soil treatment with nearly 2 tons of lime per acre. Later he reported a measure of control by applying carbon bisulfide or formaldehyde (41). In the opinion of Branas (4) the only means of controlling the disease was careful extirpation of all diseased and healthy stock to a distance sufficient to prevent the migration of Phylloxera through the soil. He pointed out that control measures in use, such as the application of carbon bisulfide, were only partially effective. Vuittenez (52) recently obtained control of court-noué by fumigating the soil with D-D (dichloropropane and dichloropropene), methyl bromide, or carbon bisulfide (listed in order of effectiveness).

SOIL-BORNE NATURE OF FANLEAF IN CALIFORNIA.—Young vines that replace recently removed diseased vines have been observed in California to have a high incidence of fanleaf. The pattern of distribution is variable: in most cases fanleaf-diseased vines are confined to discrete concentric areas, whereas on some properties the entire planting is involved.

To determine the means of transmission of California fanleaf virus, experimental plots were established in the Santa Clara Valley in a vineyard replanted only 8 years before, in which all of the replanted vines had fanleaf. In the fall of 1952 a block of vines were removed and the soil prepared for replanting. One hundred 1-year-old rootings of healthy *V. rupestris* var. St. George were planted in March, 1953. By the following spring 83% of the vines showed symptoms of fanleaf, demonstrating a high rate of spread.

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St. George was chosen as a test plant because it is extensively planted as a rootstock in the vineyards of northern California. In graft-transmission tests it had been shown that St. George is very susceptible to fanleaf: the leaves often show symptoms within 4–6 weeks after spring grafting. The symptoms are clear, distinct, and characteristic of the disease in the leaves of St. George. Early symptoms are veinlet clearing, line patterns, and flecking. The leaves later become characteristically deformed, with a wide petiolar sinus, rough surface, and elongated, irregular, marginal lobes with deep-cut marginal sinuses (Fig. 1).

In the spring of 1954, to test possible aerial spread of fanleaf, 20 rootings of St. George were planted adjacent to 20 old vines in a portion of the Santa Clara vineyard. Another 20 rootings, in steam-pasteurized non-vineyard soil in large nursery cans on a 4-inch-high wooden platform, were maintained within a few feet of the newly planted test vines and watered at intervals during the season. The next spring, the 20 nursery cans were removed to Davis, California, where they were maintained until 1957. Nineteen plants survived, and all remained healthy. In contrast, only 16 survived of the 20 St. George rootings in the vineyard, and all had fanleaf by the middle of June, 1955. This experiment shows that fanleaf did not spread through the air but was soil-borne.

Another experiment was set up in the spring of 1954. Soil was taken from the root zones of fanleaf-diseased grapevines in 2 widely separated vineyards in the Santa Clara Valley. These soils, hereinafter referred to as "fanleaf soils," were collected at the base of the vine, from an area about 10 in. across and 18 in. deep, and put separately into 3-gal. tin-lined iron nursery containers. As a control, non-vineyard Yolo fine sandy loam was collected at Davis, and is hereinafter referred to as "Davis soil."

For each treatment, nursery cans of soil were selected at random, equal numbers from each of the 2 vineyards. Preparatory to planting, each can of soil was emptied into another tar-coated 3-gal. nursery can, restoring the original top soil to the top.

Fanleaf-diseased Pinot Chardonnay vines growing on St. George rootstock were dug from one of the vineyards at the time that soil was taken. In both vineyards, cuttings were also taken from diseased vines. Healthy cuttings of *V. vinifera* varieties and rootings of St. George, 1202 *(mourvedre* × *rupestris)*, and 3309 *(riparia* × *rupestris)* used as test plants were from the University of California, Department of Viticulture, at Davis.

Table 1 shows the treatments given the different soils; the age, number, type, and variety of vine grown; the number of cans in each test; and the transmission obtained by June, 1957. Since there were no differences in transmission between the 2 vineyard soils, the results are combined in the Table.

All healthy test plants in fanleaf soil developed fanleaf symptoms by the following spring (1955). Six of 10 healthy Mission cuttings in fanleaf soil showed fanleaf symptoms by the following spring, and 3 more by the spring of 1957. Five of the 10 French Colombard cuttings in fanleaf soil showed symptoms in the spring of 1956.

Fanleaf did not develop in pasteurized fanleaf soil or in pasteurized Davis soil, nor did it transmit from fanleaf-diseased to healthy plants growing together in a nursery can in pasteurized soils.

To confirm the presence of fanleaf, buds from 3 St. George plants infected through soil were chip-grafted to 3 St. George and 3 Mission indicator plants in the spring of 1955. All 6 indicator plants developed typical symptoms of fanleaf by the spring of 1956.

SEARCH FOR VECTOR.—A search for possible vectors was begun in the spring and summer of 1955, with an examination of fanleaf soil from some of the cans. A number of organisms were found, including mites, Phylloxera, many fungi and bacteria, and several species of nematodes. Extensive tests with Phylloxera failed to transmit the virus from diseased to healthy plants. The results of these tests confirm the observations and tests of others (25, 28, 52).

Nematode species most consistently found were *Xiphinema index* Thorne & Allen (48) and *Criconemoides xenoplax* Raski (38); less frequent were *X. americanum* Cobb and species of *Pratylenchus*, *Meloidogyne*, and *Hemicriconemoides*.

Tests of nematodes as possible vectors were pre-

TABLE 1.—*Transmission of fanleaf to healthy grapevines in nursery cans of fanleaf soil, but not in pasteurized soil*

Soil, treatment, and planting	No. of cans Planted	No. of cans Showing transmission
Fanleaf soil		
1 healthy rooting, St. George	20	20
1 healthy rooting, rootstock No. 1202	5	3[a]
1 healthy rooting, rootstock No. 3309	5	5
4 healthy cuttings, Mission	10	9
4 healthy cuttings, French Colombard	10	5
1 fanleaf rooting, 1 Pinot Chardonnay on St. George, and 1 healthy rooting on St. George	20	20
Fanleaf soil steam pasteurized		
1 healthy rooting, St. George	10	0
4 healthy cuttings, French Colombard	10	0
3 fanleaf cuttings, Pinot Chardonnay, and 3 healthy cuttings, French Colombard	10	0
Davis potting soil steam pasteurized		
1 healthy rooting, St. George	10	0
4 healthy cuttings, French Colombard	10	0
3 fanleaf cuttings, Pinot Chardonnay, and 3 healthy cuttings, French Colombard	10	0

[a] The other 2 plants died, of undetermined cause, during the first season.

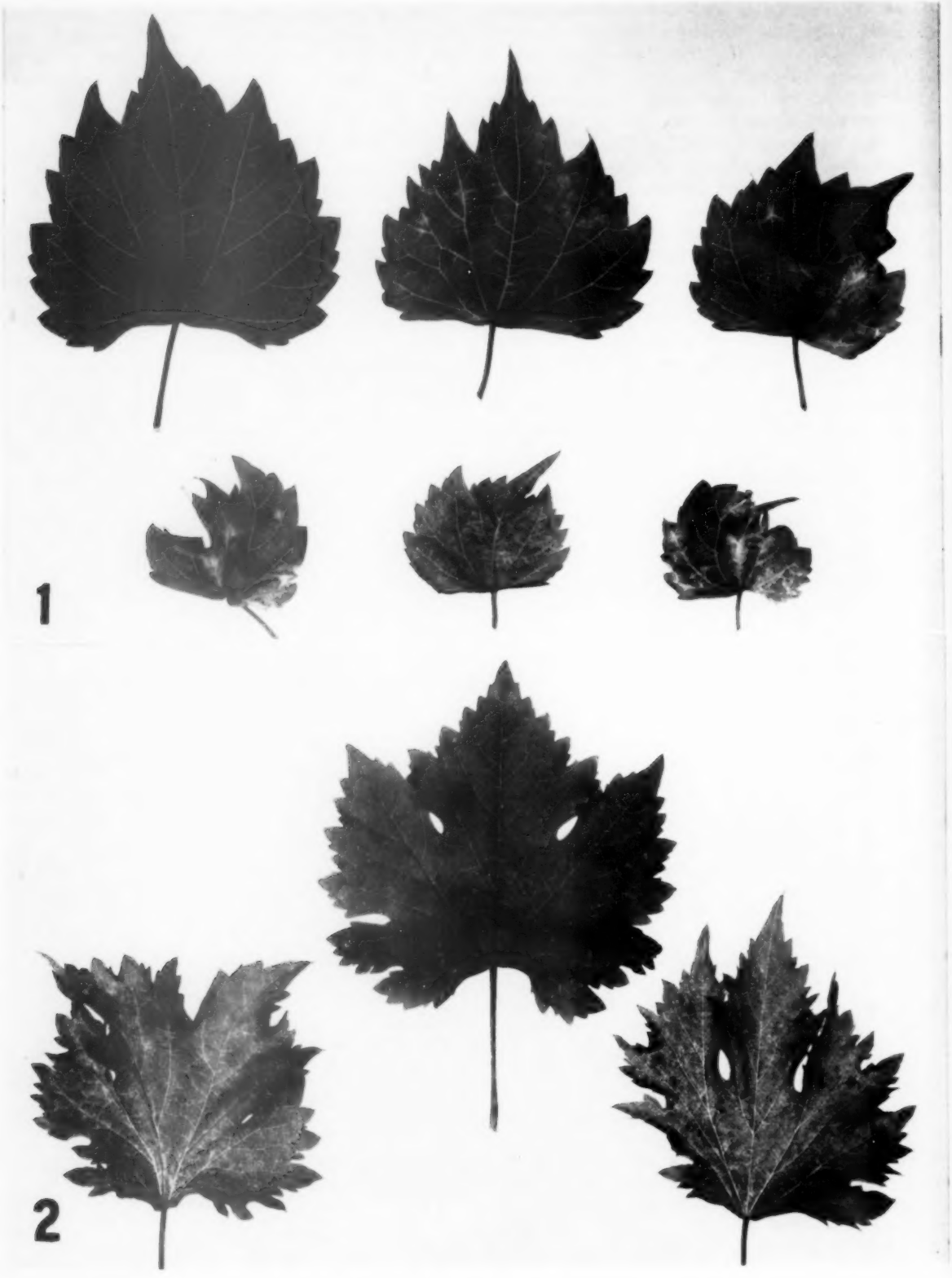
1
2

TABLE 2.—*The number of X. index and C. xenoplax used to infest soil and roots of healthy Mission vines in August, 1955, the number recovered after 22 months, and the season plants first showed symptoms*

Sample and pot no.	No. of nematodes in inoculation		No. of nematodes recovered after 22 months		Fanleaf-diseased vines	Year symptoms first observed
	X. index	C. xenoplax	X. index	C. xenoplax		
5 control plants	0	0	0	0	(Healthy)	
1	40	+[a]	0	6,000	(1958)	
2	0	—	0	0		
3	30	+	3,000	1,100		
4	0	—	0	2,100		
5	40	+	500	1,900		
6	40	—	5,508	660	Fanleaf	1956
7	50	—	640	160		
8	0	—	6,188	1,925		
9	40	60	4,140	4,980	Fanleaf	1956
10	0	720	0	19,008	Fanleaf	1958
11	0	1,550	0	12,165		
12	50	990	0	8,170	Fanleaf	1958
13	60	110	0	891	Fanleaf	1956
14	0	1,250	0	72,750		
15	30	100			[b]	
16	0	1,270	4,335	4,335		
17	0	1,280	0	8,200		
18	50	110	600	4,250	Fanleaf	1958
19	0	760	0	19,734	Fanleaf	1958
20	10	650	200	13,000		

[a] *C. xenoplax* observed present but not counted are marked +. Samples in which adult nematodes were not observed are marked —. Starting with sample 9, all of this species were counted and recorded.
[b] Plant dead in 1956, cause undetermined.

pared as follows: Soil samples of about 500 g each were taken from the root zones of fanleaf-diseased plants, either in the vineyard or in experimental nursery cans. Each sample of soil was washed through a 40-mesh screen and a 200-mesh screen in series. Nematodes as well as debris retained on the 200-mesh screen were rinsed with tap water, washed from the screen, and held in water, which was then adjusted to 100 ml. From the water, thoroughly stirred, a 10 ml aliquot was withdrawn for identification and count of nematodes, and the remaining 90 ml of water and screenings were poured over the root and soil clump of a healthy Mission grapevine rooting that had been removed from a 6-in. clay pot. Each vine was then returned to the pot and held in a greenhouse for incubation. Records were kept of the numbers of *X. index* and *C. xenoplax* (though not of other species) because only these 2 species were found consistently in the samples of fanleaf soil. During the dormant season (November-February) the vines were removed to a lathhouse.

In August, 1955, 20 healthy Mission vines were thus infested with *X. index*, *C. xenoplax*, and occasionally other species as listed above, obtained from fanleaf soil that had transmitted fanleaf. Five undisturbed Mission vines in 6-in. clay pots, maintained as control plants, were placed at random among the infested pots. All test vines were examined for fanleaf in May, 1956, June, 1957, and March, 1958. Total numbers of *X. index* and *C. xenoplax* in each pot were determined in June, 1957, 22 months after infesting. Table 2 lists the number of nematodes administered and recovered, the number of plants that developed fanleaf, and the season when symptoms were first observed. The symptoms of fanleaf that developed in the leaves of Mission grape in these tests (Fig. 2) are the same as those that show in diseased vines in vineyards (20).

Though the numbers of nematodes introduced into each pot, especially of *X. index*, were low, recoveries were generally very high. Adult *X. index* were introduced into 10 of the 19 plants that lived. They were recovered from 7 of these plants, and also from 4 plants that apparently had not received adult nematodes. Their absence from 3 plants that had received *X. index* (according to aliquot samples) indicates that they did not always survive and build up under

FIG. 1–2. Fig. 1. Fanleaf symptoms in leaves of *V. rupestris* var. St. George. The leaves are from plants in the experiment on transmission through fanleaf soil (Table 1); upper left, a normal leaf; the other leaves show chlorosis, vein clearing, and deformity typical of first-season symptoms in the variety.—Fig. 2. Fanleaf symptoms in leaves of *V. vinifera* var. Mission. The leaves are from plants growing in soil infested with nematodes from fanleaf soil (Tables 2 and 3); center leaf is normal from one of the control vines; the other 2 leaves have translucent veins and are deformed, characteristic of the second and later seasons of disease in the variety.

TABLE 3.—*The number of X. index from fanleaf soils used to infest roots of grape growing in sandy loam soil in May and June, 1956, the number of recovered, the plants developing fanleaf, and the season symptoms were first observed*

Variety	Infesting population 1956	Recovered population 1957	Fanleaf-diseased	Year symptoms first observed
Soil from Vineyard S				
Mission	100	26,500	**	
Mission	100	2,300	*	
Mission	100	3,800		
Mission	100	36,500	*	
Mission	76	11,500		
Soil from Vineyard B				
Mission	100	8,400	Fanleaf	1958
Mission	100	11,600		
Mission	100	5,900		
Mission	100	9,400		
Mission	110	0		
Soil from other experiments[b]				
Mission	105	35,000	Fanleaf	1958
Soil from Vineyard A				
Mission	100	300		
Mission	144	5,400	Fanleaf	1957
Soil from other experiments				
St. George	10	4,200		
St. George	100	6,765	*	
St. George	664	30,096	Fanleaf	1958
St. George	10	1,500	*	
St. George	100	6,336	Fanleaf	1958
St. George	1,000	3,029	*	

[a] Plant dead, 1958, of undetermined cause.

[b] Source of nematodes was a fanleaf plant from some other experiment in which *X. index* population was fairly high.

this treatment. Determination of *C. xenoplax* numbers that were introduced with screenings was begun with sample 9. This nematode was recovered from all pots into which it was known to have been introduced.

Three plants in this series developed fanleaf within 9 months after infestation, and 4 additional plants showed fanleaf symptoms after 32 months (Fig. 2). Of these 7 pots, 5 had received *X. index* (which was recovered from 3), and 6 had received *C. xenoplax* (which was recovered from all 7).

In another test, 15 soil samples of about 500 g each were taken from a nursery can of fanleaf soil that had high counts of both *X. index* and *C. xenoplax*. Screenings from each sample, prepared as before, were used to infest the roots of a Mission vine growing in a 6-in. clay pot. These screenings were not examined for nematode numbers. The plants were infested in late August, 1955. Five uninfested Mission plants were maintained as controls. Symptoms of fanleaf were observed on 4 of the 15 infested plants by June, 1956, and on 3 additional plants by June, 1957. To force early growth, the plants were moved into a greenhouse in February, 1958; by March 24 there were 6 additional plants with fanleaf, making a total of 13 transmissions in 15 pots infested. The 5 healthy control Mission vines dispersed among the plants remained healthy, an indication that the slow development of symptom expression over 3 seasons was not due to a spread of contamination from pot to pot. The delay in symptoms might have been caused by slow plant growth: with the roots pot-bound, it may have taken this time for the virus to move from the roots into the top. It is also possible that only a small amount of virus was introduced into the plants. Nematode counts made after 22 months showed that all 15 infested pots had populations of *X. index*, ranging from 220 to 5280; 13 of the 15 pots had populations of *C. xenoplax*, ranging from 90 to over 10,000.

The tests show that a vector of fanleaf virus was retained on the 200-mesh screen, and indicate that the vector was *X. index* or *C. xenoplax*. Since soil debris, mostly organic matter, was caught in the screenings, and since the nematodes themselves probably were contaminated with other microorganisms, further tests were conducted.

In May and June, 1956, nematodes were washed from fanleaf soil, recovered in water, and hand-picked under a binocular microscope into small quantities of distilled water in beakers. In this way the specimens were separated and washed free of most debris and soil particles. Separate populations, varying from 10 to 1000 each, of the 2 species were then poured over the roots of 1- and 2-year-old plants of healthy Mission and St. George.

Table 3 shows the soil source of *X. index*, the numbers administered and recovered, the grape varieties tested, the number of plants that developed fanleaf, and the season when symptoms were first observed.

The populations of *X. index* increased in all but 1 of the pots of test grapes. Only 1 vine in this series showed fanleaf symptoms by 1957; after the plants were forced in the greenhouse, in the spring of 1958, 4 more developed fanleaf. Six plants died in 1958, probably as a result of high nematode populations.

In the manner described above, 13 plants were infested with *C. xenoplax* populations that varied from 10 to 1000 individuals. In this series 1 plant died of unknown causes. The other 12 pots at 10 months contained *C. xenoplax* populations of 150–15,500, and 1 pot contained 182 *X. index* besides 800 *C. xenoplax*. The mixture of nematodes could represent contamination. One of the plants in the series developed fanleaf. *C. xenoplax* but not *X. index* were recovered from the soil around this plant.

In another experiment, in May of 1956, small St. George cuttings, rooted in steam-pasteurized quartz sand in 6-in. clay pots and watered with nutrient solution, were infested with adult *X. index*, hand-picked from fanleaf-soil washings and suspended in water, as previously described. Ten nematodes were administered to each of 4 plants, 100 to each of 4, and 1000 to each of 4. A similar series was set up

with *C. xenoplax*. Six uninfested plants were maintained at random among the test plants, as controls.

In March, 1957, 3 of the plants infested with 1000 *X. index* had fanleaf; the fourth plant had died. No symptoms had developed a year later in the plants infested with 10 or 100 *X. index*, the 12 plants infested with *C. xenoplax*, or the 6 control plants. No living nematodes were recovered from any of the 30 plants. The loss of nematodes and failure of fanleaf in infested plants could be explained by the plugging of drains during the winter, while the plants were dormant. They were waterlogged for some time, the roots rotted badly, and several plants died.

The 3 St. George plants that showed fanleaf symptoms were approach-grafted to healthy St. George plants in sandy loam in 6-in. pots. After 5 weeks the approach grafts were cut, leaving a portion of the plant being indexed attached to the test plant. In March, 1958, 1 year after the grafts were made, all 3 indicator plants had fanleaf (Fig. 3).

By the spring of 1957 *X. index* appeared from the experiments to be a vector of fanleaf virus, whereas *C. xenoplax* was questionable. It was therefore decided to concentrate on *X. index* in 1957.

In June, 1957, adults and fourth-stage larvae of *X. index* were screened from fanleaf soil, individually hand-picked into separate lots of 100, 250, 500, and 1000 in sterile water. Each lot was washed 3 separate times by settling the nematodes in about 30 ml of water and decanting the water. The decanted water, referred to herein as "supernatant," was collected for each lot and used in the experiment.

Test plants were St. George, from small cuttings rooted and grown about 4 months in quartz sand watered with nutrient solution. As the cuttings were transplanted to sandy loam or coarse plaster sand in 4-in. clay pots, the roots of each plant were infested by pouring around them a suspension containing a known number of nematodes. Five plants were infested at each population level.

To determine if the supernatant carried an entity capable of transmitting the virus, the wash water from each lot was similarly poured around the roots of a test plant. Two lots of control plants were handled identically except that clean water was poured around the roots. One control lot was planted in sandy loam and the other in coarse plaster sand.

In September and October of 1957, because plants in some of the lots infested with nematodes were dwarfed and the leaves badly burned, the nematodes were washed from the roots and the plants repotted in clean soil. Nematode populations had increased on all plants, and the roots were badly damaged, knotted, and distorted.

All plants were grown in a greenhouse until November 1, 1957, when they were moved to a lathhouse for wintering. On January 17, 1958, they were returned to the greenhouse for forcing. During the experiment the plants were watered with nutrient solution. Table 4 shows nematode populations and fanleaf transmission for the 12 lots.

By early February, 1958, most nematode-infested plants were very weak and some were dead, whereas the control plants and most of those that had re-

FIG. 3. Shoots of *V. rupestris* var. St. George; left, from a normal plant; center, shoot from an indicator plant that was approach-grafted to a plant with fanleaf that had received 1000 *X. index* from fanleaf soil; right, shoot from an indicator plant chip-grafted with wood from a plant with fanleaf that had contracted the virus from another fanleaf-diseased plant in a pot infested with *X. index* from healthy grape roots (Table 5).

TABLE 4.—*Number of St. George plants that developed fanleaf when infested with different numbers of X. index from fanleaf soil, the infesting and the minimum and maximum populations recovered, and the effect of the wash water from rinsing nematode populations on the development of fanleaf*

Lot no.	Treatment	No. plants	Soil used	X. index numbers: Plants infested	Infesting	Recovered	Healthy	Fanleaf	Dead
1	Control	5	Sandy loam	0	0	0	5	0	0
2	Control	5	Sand	0	0	0	5	0	0
3	Nematodes	5	Sandy loam	5	100	3,200–6,200[a]	0	3	2
4	Supernatant[b]	5	Sandy loam	1	0	0–3,900	5	0	0
5	Nematodes	5	Sandy loam	5	250	2,800–3,400	0	4	1
6	Supernatant	5	Sandy loam	3	0	0–24,000	5	0	0
7	Nematodes	5	Sandy loam	5	500	900–11,200	0	2	3
8	Supernatant	5	Sandy loam	3	0	0–17,100	5	0	0
9	Nematodes	5	Sand	5	1,000	700–4,900	0	5	0
10	Supernatant	5	Sand	1	0	0–100	4	1	0
11	Nematodes	5	Sandy loam	5	1,000	7,350–11,066	0	3	2
12	Supernatant	5	Sandy loam	1	0	0–26,800	4	1	0

[a] The minimum and maximum populations of *X. index* in the series of 5 pots 3½ months after infesting. The plants in lots 3, 5, 7, 9, and 11 were so weakened that the nematode populations were removed at this time.

[b] In each case, the "supernatant" refers to the water used to wash the previous lot of nematodes. They were washed in water 3 separate times, and the water was collected and poured over the root mass of the corresponding separate test plant.

ceived supernatant were advanced in growth. By February 17, some of the nematode-infested plants had mottled leaves and showed other fanleaf symptoms: vein clearing, line pattern, and chlorotic rings; other plants were so badly dwarfed that it was difficult to read symptoms with accuracy. Plants showing each response were indexed by grafting stem chips and bud chips into healthy St. George indicator plants. A month later, the indicator plants had fanleaf, thus establishing the graft-transmissibility of the fanleaf symptoms in these plants (Fig. 3).

Some pots that had received supernatant liquid contained high populations of *X. index*, and 1 plant each in lots 10 and 12, which had fanleaf, also contained *X. index* (Table 4). Since the pots were well spaced and all control plants remained free of nematodes, it is possible that nematodes or eggs were introduced with the supernatant. It is also possible that those which developed fanleaf may have received viruliferous nematodes or even viruliferous nematode eggs.

TRANSMISSION BY X. INDEX COLLECTED FROM HEALTHY GRAPE AND FIG ROOTS.—Soil was collected from the root zone of a Tokay grape in an old vineyard near Lodi, California. The vine, indexed on St. George, had been found free of fanleaf virus. *X. index* were screened from the soil, individually hand-picked, washed, and suspended in water. To test fanleaf transmission by these nematodes, healthy and diseased rootings were planted together in pasteurized sandy loam soil, 1 pair per 6-in. pot, and *X. index* adults suspended in water were poured on their roots.

Test plants were 1-year-old healthy St. George rootings. The fanleaf-diseased vines were 1-year-old St. George rootings made from the green shoot tip of fanleaf-diseased vines. All cuttings had been rooted in pasteurized sand and grown in pasteurized soil to keep them free of contamination.

Twenty pots of paired vines were thus infested with *X. index* from non-fanleaf soil; 10 pots contained paired vines without nematodes; 25 pots containing healthy St. George plants alone were infested with 10 *X. index* each; and 10 pots contained healthy control plants alone without nematodes.

Another experiment was similar in all respects except that the nematodes were screened from soil dug from the roots of old fig trees, the type host for *X. index* Thorne & Allen (48). These results also are shown in Table 5.

Both experiments were started in July, 1957. The plants were maintained in the greenhouse until November 1, moved to a lathhouse for the winter, and returned to the greenhouse on January 17 to force new growth.

Table 5 shows nematode infestation and recovery, with the findings on fanleaf transmission.

All control healthy plants in uninfested pots, singly or paired with fanleaf plants, remained healthy and free of nematodes. In all infested pots the 10 original *X. index* increased to hundreds and thousands. Healthy St. George plants infested with *X. index* remained normal-appearing when planted alone, but when they were potted with fanleaf-diseased plants, symptoms developed in 11 of the 20 plants infested with *X. index* from the Tokay roots, and in 5 of the plants infested with *X. index* from fig roots. The plants showing symptoms were indexed by chip grafts and inarching to St. George indicator plants, which developed fanleaf in 4–6 weeks.

Besides showing that *X. index* is a vector that can move fanleaf virus from diseased to healthy plants in the same container, these experiments show that *X. index* from either figs or grapes can build high populations on St. George grape roots.

DISCUSSION.—Aerial spread of fanleaf did not occur in any of the experiments; neither did the pattern of distribution and slow concentric spread indicate airborne disease.

The experiments demonstrating the soil-borne nature of fanleaf are in agreement with the reports of Petri (33, 34, 35) and Ravaz (40, 41).

The fact that, in 3 years, fanleaf did not spread through pasteurized soil from diseased to healthy plants in the same container discounts the possibility that the virus spreads by root grafts and by contact with inert soil particles. If the fanleaf virus were capable of being transmitted to grape roots directly by contact with inert soil particles, then it seems likely, though not certain, that the virus would have moved from diseased vines to soil particles and on to healthy vines in 3 years. During this period, the virus should have been released to the soil by decaying grape root tissue and could have been picked up by the roots of healthy plants growing in the same container. The failure of the virus to transmit from diseased to healthy plants in pasteurized soil and the transmission of fanleaf to vines in fanleaf soil within 10 months indicated that a biological entity must serve as a vector.

Since Phylloxera were known to be present in the soils where fanleaf was spreading, it appeared to be a likely vector of the virus; however, many tests conducted with them were all negative, in agreement with the results of others (25, 28).

Nematodes were found consistently in samples of soil from the root zone of fanleaf-diseased vines. Previously, no plant nematodes have been demonstrated to be vectors of plant viruses, but in animal virus studies Shope (43, 44, 45, 46) has shown that the swine lungworm, an intermediate host of the swine influenza virus, will transmit the virus to swine, and that the virus will carry through the egg of the lungworm.

The first transmission tests with nematodes as probable vectors were exploratory. It was fully realized that the nematodes, as well as other matter in the screenings, might contain microorganisms that could also be vectors of virus. However, the recovery of *X. index* and *C. xenoplax* from the soil in the majority of the containers where test plants developed fanleaf, together with their consistent presence in soil samples from diseased vines in different vineyards, was considered very strong evidence that these nematode species were probable vectors of the fanleaf virus; therefore, to advise others interested in the mode of transmission of fanleaf virus of these results and to encourage them also to test nematodes as vectors, an oral report on the preliminary experiments was given for the authors by Professor A. J. Winkler at the 1956 conference on infectious degeneration, at Verona, Italy (31).

Symptoms are slow to develop in the top when fanleaf is inoculated into the roots; they usually show only in new growth after a dormant period. Once symptoms show in the top they persist. Summer pruning failed to induce symptom development in most cases. The relatively low numbers of plants with symptoms in the first season and the slow rate of development of symptom expression could be explained by a very slow movement of the virus from the roots to the tops of the plants (3, 15, 37).

Since fanleaf developed in a pot infested with hand-picked *C. xenoplax* from fanleaf soil, additional tests with this species are needed to confirm that it might also be a vector of the virus. Tests with other nematodes are also needed.

The experiments started in the summer of 1957 included *X. index* from 3 sources: 1) fanleaf soil; 2) soil from the root zone of normal grapevines, *Vitis vinifera* var. Tokay, that indexed normal on St. George; and 3) soil from the root zone of fig trees. The results of the experiments with these nematodes (Tables 4 and 5) are conclusive.

Even though the methods used in securing and handling these nematodes do not exclude the possibility that other microorganisms might have been transferred along with them, it is unlikely that the nematodes from normal grape and from fig were in each case carrying microorganisms that would be a vector of the virus. Further, only 10 nematodes, hand-picked and washed, were used to infest each pot. Under these conditions it seems unlikely that other microorganisms were a factor in these tests.

In these experiments, the fact that fanleaf transmission to healthy St. George occurred only in pots of fanleaf-diseased and healthy St. George that were infested with *X. index* is very good proof that *X. index*

TABLE 5.—*Transmision of fanleaf from diseased to healthy St. George plants by X. index nematodes collected from the root zone of healthy grape and from the root zone of fig*

Rootings planted in each pot	No. replications	No. X. index				
		Source	Infesting	Recovery	Healthy	Fanleaf	Dead
1 healthy	10	Grape	0	0	10	0	0
1 healthy	25	Grape	10	2,300–19,000	25	0	0
1 healthy and 1 fanleaf	10	Grape	0	0	10	0	0
1 healthy and 1 fanleaf	20	Grape	10	1,600–21,460	8	11	1
1 healthy	10	Fig	0	0	10	0	0
1 healthy	25	Fig	10	3,700–39,960	25	0	0
1 healthy and 1 fanleaf	10	Fig	0	0	10	0	0
1 healthy and 1 fanleaf	19	Fig	10	244–17,000	12	5	2

transmitted the fanleaf virus.

This report is believed to be the first to show that nematodes are vectors of one of the soil-borne plant viruses.

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ASPARTIC AND GLUTAMIC ACID AS SELF-INHIBITORS OF UREDOSPORE GERMINATION[1]

Eugene M. Wilson[2]

SUMMARY

Chromatographic analysis of the aqueous diffusates from the uredospores of bean rust showed the presence of 8 distinct substances that reacted with ninhydrin. The 3 predominant substances were beta alanine, glutamic acid, and aspartic acid. Quantitative chromatography showed that the latter 2 amino acids were in sufficient quantities to account for the self-inhibition of uredospore germination. The self-inhibitors of bean rust uredospores were present in tissue surrounding rust pustules on bean leaves. These inhibitors formed a concentration gradient with highest concentration in the tissue closest to the pustule. They protected against reinfection of tissue close to the rust pustules. Inhibitor-depleted uredospores of the bean rust fungus survived in storage at higher temperatures for longer periods than did untreated uredospores. Optimum temperature for germination was 20°C for inhibitor-depleted bean rust uredospores, and 16°C for untreated uredospores from the same harvest. Protection against reinfection due to an initial infection of bean rust was reduced by lowering the temperature of incubation. At 16°C there appeared little, if any, protection due to a preceding infection.

Frequency of germination of bean rust uredospores and reinfections of rusted bean leaf tissue were increased with 50–100 ppm of coumarin or 9.12 ppm of 2,4-dinitrophenol. Indole-3-acetic acid (up to 10 ppm) or gibberellic acid (up to 1000 ppm) did not increase reinfection of rusted tissue or the germination of uredospores. Self-inhibitors of germination were detected in the uredospores of 5 rust species, but not of *Cronartium ribicola* Fischer. There was a correlation between the number of bean rust uredospores per unit of substratum and the length of germ tubes, which was not explainable in terms of self-inhibitors.

Some inconsistent results from germination studies of rust uredospores have been recently explained on the basis of self-inhibitors (1, 2, 4, 5, 6, 8, 9, 10). Whether these inconsistent results are due to self-inhibitors alone or to some other factor in addition has not been proven, nor have the self-inhibitors themselves been isolated or identified.

Self-inhibition of uredospore germination in rust fungi has been associated with an adverse effect due to concentration of spores (1, 4, 8, 9, 10). Allen (1) found that variation in uredospore germination in *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. was caused by a germination inhibitor found in aqueous diffusates from the uredospores. Wilson (7) reported a dosage-response with aqueous diffusates from the uredospores of bean rust, *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth., against infection with bean rust.

The present study was made to identify the self-inhibitors in bean rust uredospores, explain their activity, determine their presence or absence in the uredospores of other rust species, explain certain variations in spore germination, and correlate the presence of these inhibitors with protection against reinfection by the same rust.

METHODS AND MATERIALS.—Uredospores of *Cronartium ribicola* Fischer, *Puccinia graminis* f. sp. *tritici* race 15-B, *Puccinia helianthi* Schw., *Puccinia polygoni-amphibii* Pers. var. *persicariae* (Strauss) Arth., *Puccinia carthami* Cda., and *Uromyces phaseoli* var. *typica* were harvested and stored in Petri dishes at 4°C. The uredospores were propagated under greenhouse conditions, except those of the Polygonum rust, which were harvested from infected weeds during the dry season. Watering the plants at the soil level proved to be important in obtaining harvestable uredospores and in preserving the self-inhibitors in the uredospores. The uredospores were harvested by shaking the rusted leaf over a waxed paper or a glass jar.

The criterion for germination was the production of a germ tube longer than the diameter of the uredospore. Percentage germination was the average of 3

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TABLE 1.—*The effect of temperature and spore concentration on germination of bean rust uredospores from a single harvest*

°C	Spores/cm² (× 10⁴)	Average % germination
22.0	11.22	22.8
	14.98	0.1
	21.90	6.0
	29.80	1.3
19.5	2.56	18.5
	5.43	22.2
	28.30	5.3
	35.03	3.4
16.0	6.66	26.6
	12.55	25.9
	20.28	8.9
	43.50	5.1

separate and random counts of 100 spores each. All experiments were repeated at least 3 times.

To obtain the self-inhibitors, 2 g of uredospores suspended in 1 liter of distilled water were distributed equally among 6 open halves of Petri dishes (15 cm in diameter). Four 1×6-in. filter paper strips (Whatman No. 1), each creased 1 in. from the top and pointed at the other end, were hung from the rim of the Petri dish as wicks. In room conditions free of strong air currents the self-inhibitors accumulated in the pointed tips of the paper wicks. The spores in the dish were dry after about 36 hours. The point of the dry paper wick was cut off about 1 in. from the tip, since tests showed that the self-inhibitors concentrated in this area. To get a concentrated sample of these self-inhibitors, many of these paper points were eluted with distilled water, and the resulting solution was lyophilized. The sample was first frozen by immersion in a dry-ice–Cellosolve bath and then dried about 16 hours in a partial vacuum. Uredospores that remained in the dish appeared to be relatively free of self-inhibitors as evidenced by their germination *en masse* on water agar. They are referred to as inhibitor-depleted uredospores.

An agar diffusion method was used as a bioassay for the inhibitors in uredospores and in rusted tissue. A rust pustule, a clump of uredospores, a ring of adjacent tissue surrounding a rust pustule, or other tissue of rusted plants was placed on the surface of a thin layer of 2% water agar (8 ml in a 9-cm Petri dish). This was then covered with an equally thin layer of water agar by means of a section lifter. After 3 hours to allow the inhibitors to diffuse into the water agar, the exposed surface was dusted with uredospores of the assay rust. In some tests, inhibitor-depleted uredospores were used. The spores were sparingly distributed over the agar surface to ensure against the possible effect of the self-inhibitors from the assay rust spores themselves. These plates were held in the dark at 21°C. After 6 hours the resulting zone of inhibition could be seen with a dissecting microscope at ×10 magnification; and after a longer period, with the unaided eye. The resulting zone of inhibition was directly proportional to the concentration of the inhibitors in the sample and the time allowed for their diffusion into the agar. At room temperature a diffusion period of 3 hours was found suitable for these studies.

RESULTS.—*Relation of temperature and spore concentration on per cent germination.*—Yarwood (8) reported that, on water agar in closed Petri dishes, there was a 3.35-fold increase of uredospore germination when the number of spores was decreased from 19,000 to 1100 spores per cm².

During this study the relation was determined between number of uredospores on water agar (10 ml in 9-cm Petri dishes), temperature of incubation, and per cent germination. Bean rust uredospores from the same harvest were dusted uniformly onto the surface of the water agar with a DeVilbiss No. 119 insufflator. To determine the influence of spore concentration, some surfaces were more heavily seeded with uredospores than were others. The spores were incubated 7 hours in the dark at either 16, 19.5, or 22.0°C (Table 1).

Repetitions of these experiments showed more than a 4-fold increase in germination at all temperatures tested when uredospore numbers were reduced from 43.5×10^4 per cm² to 6.66×10^4.

In similar experiments, with other samples of uredospores of the bean rust fungus and with uredospores of the wheat rust fungus, this self-inhibition through increasing uredospore numbers was not observed. The temperature and humidity at which the uredospores developed apparently influenced the phenomenon. Pinto bean plants were inoculated with the bean rust fungus, incubated, held 4 days at 24°C, and then held at 18°C at 60% relative humidity until the uredospores became free in the pustules. Increasing the number of these uredospores per unit area of water agar surface did not decrease per cent ger-

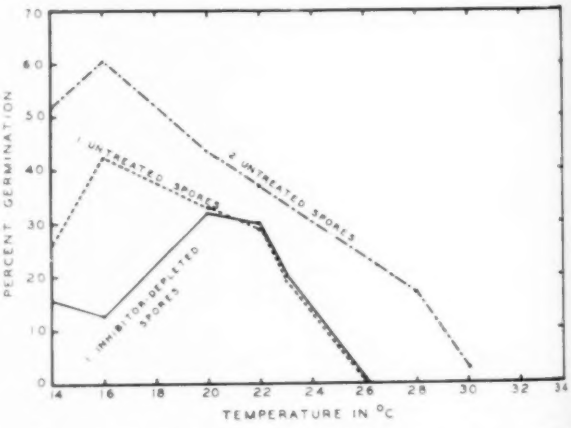

FIG. 1. The influence of temperature on per cent of germination of inhibitor-depleted and untreated bean rust uredospores from the same harvest (1), and of untreated uredospores from a different harvest (2). The inhibitor-depleted spores have a higher optimum temperature.

mination even at a concentration as high as 2.66×10^5 spores per cm^2. This indicated that these spores contained little or no inhibitor of germination. Similar results were obtained with the wheat rust fungus when the uredospores developed at a relatively low temperature (16°C) and at a high humidity (80–90%). Such uredospores showed 90–95% germination at spore concentrations as high as $1.28\times10^6/cm^2$.

To determine the influence of temperature on the germination of inhibitor-depleted and untreated uredospores of bean rust, spores were dusted uniformly on the surface of 10 ml of water agar in Petri dishes and incubated 7 hours in the dark at different temperatures. With spores of the same harvest the optimum temperature was 20°C for germination of inhibitor-depleted uredospores, and 16°C for untreated uredospores. There was only a non-significant variation in temperature of germination of untreated spores from one harvest to another (Fig. 1).

The influence of spore concentration on germ tube length.—For bean rust uredospores, Yarwood (9) reported a correlation between the number of bean rust uredospores per unit of substratum, on the one hand, and percentage germination and length of germ tube on the other. Ezekiel (3) reported a correlation between the increasing number of uredospores per unit of substratum and the increasing length of germ tubes of *Puccinia graminis* var. *tritici*. Yarwood's (9) report was corroborated during the present study.

In the present study short germ tubes were produced by bean rust uredospores at certain concentrations of the self-inhibitors. When the concentration of the spores was increased until almost all spore germination was inhibited, there infrequently occurred germ tubes that were much longer than those of untreated uredospores. The relation between number of uredospores per unit of substratum and length of germ tubes could not be explained in terms of the self-inhibitors. It was found, however, that glutamic acid and beta alanine were present in the aqueous diffusate from bean rust uredospores, and at 10 ppm in water agar these materials resulted in a 4-fold increase in germ tube length. This stimulation did not occur at either 1 or 100 ppm.

Experiments on the relation of spore concentration to germ tube length on water agar (10 ml in 9-cm Petri dishes) produced the following results: at 10^3–10^4 bean rust uredospores per cm^2, average germ tube length was 36.88 μ; at 10^4–10^5, 52.6 μ; and at 10^5–10^6, 75.7 μ. However, per cent germination at 10^5–10^6 was only 1 spore in 10^6.

Aspartic and glutamic acids as self-inhibitors.—Paper chromatography was used for identifying the amino acids present in the aqueous solution of the uredospore self-inhibitors. A small spot (1 cm in diameter) 4 in. below one edge of a sheet of Whatman No. 1 filter paper (18×22 in.) was repeatedly wet with this solution and allowed to dry. The final quantity of self-inhibitors placed in the spot varied with the concentration of the solution and the volume applied to the spot. This one-dimensional chromatogram was developed either with n-butanol, acetic acid, and water (4:1:5 by volume) or with freshly distilled phenol. Chromatograms were developed at room temperature for 16–18 hours. The papers were dried 6–8 hours, and sprayed with ninhydrin to determine the location of amino acids.

Four distinct spots appeared on the chromatogram developed with the phenol solvent, but 8 distinct spots appeared on the chromatogram developed with the n-butanol, acetic acid, and water solvent. Solutions of chemically pure amino acids were similarly spotted on the same papers to serve as a basis for identifying the amino acids.

The 3 upper spots (spots 1, 2, and 3) were larger and darker than the other spots found on the papergrams developed in the n-butanol, acetic acid, and water solvent. Comparative R_F values with this and the phenol solvent served as the basis for identifying the amino acids in these 3 spots as aspartic acid, glutamic acid, and beta alanine, respectively (Table 2).

Uredospore germination was inhibited by either aspartic or glutamic acid in water agar. The dosage response curves for these amino acids are given in Fig. 2. Neither aspartic nor glutamic acid at 1,000 ppm caused any perceptible phytotoxicity when sprayed onto the primary leaves of bean plants.

Range of inhibitory activity.—On a potato-dextrose-peptone agar surface that had been seeded with bacteria, filter paper disks containing an aqueous solution of the rust self-inhibitors produced no inhibition zone with the following bacteria: *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn., *Corynebacterium michiganense* (E. F. Sm.) Jens., *Erwinia amylovora* (Burr.) Winslow et al, *Mycobacterium phlei planum* Haag., *Pseudomonas fluorescens* Migula., *Pseudomonas syringae* van Hall, or *Xanthomonas juglandis* (Pierce) Dowson.

In similar experiments, with a potato-dextrose-agar medium, the growth of *Fusarium oxysporum* Schlecht.

TABLE 2.—R_F *values of free amino acids leached from uredospores of the bean rust fungus and of reference amino acids on the same chromatogram. Note the close correlation between the* R_F *values of the first 3 spots and those of the 3 reference amino acids*

Number of spot or reference amino acid	R_F value in 2 solvent systems	
1	0.20[a]	0.04[b]
2	0.31	0.09
3	0.62	0.15
4	0.81	0.23
5		0.28
6		0.34
7		0.40
8		0.47
D-L aspartic acid	0.21	0.04
L glutamic acid (hydrochloride)	0.33	0.09
Beta alanine	0.61	0.15

[a] Phenol and water (80% phenol).
[b] Butanol, acetic acid, and water (4:1:5).

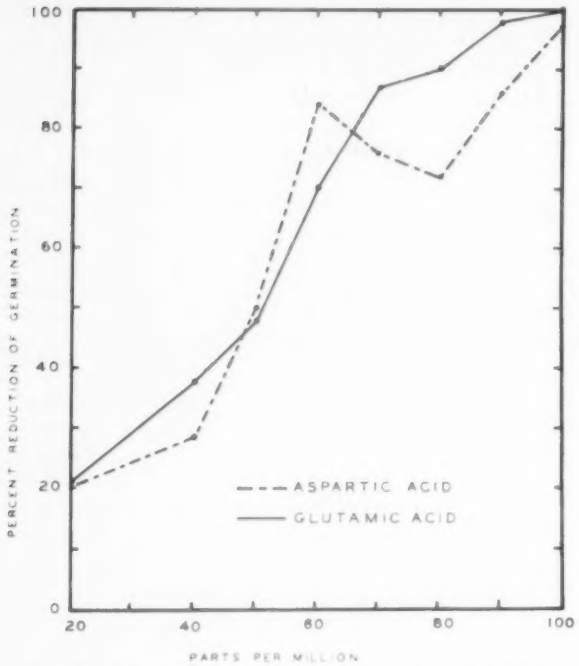

FIG. 2. Per cent reduction of germination of inhibitor-depleted uredospores of bean rust due to different concentrations of pure aspartic and glutamic acids.

f. *lycopersici* Sacc., *Glomerella cingulata* (Stonem.) Spauld. & Schrenk, or *Ustilago zeae* (Beck.) Ung. was not inhibited by the self-inhibitors.

The germination of seeds of oat (*Avena sativa* L.), wheat (*Triticum sativum* L.), or pea (*Pisum sativum* L.) was not inhibited by concentrations of rust self-inhibitors that were sufficient to inhibit the germination of bean rust uredospores. At these concentrations the bean rust self-inhibitors also inhibited the germination of uredospores of the following rusts: *Cronartium ribicola* Fischer, *Puccinia graminis* var. *tritici*, *Puccinia carthami*, *Puccinia helianthi*, *Puccinia polygoni-amphibii persicariae*.

The distribution of germination inhibitors in the tissue of rust-infected leaves.—Yarwood (8) reported that rust-infected bean leaves were protected against reinfection by the bean rust fungus. He suggested that this protection was due to a volatile self-inhibitor of the germinating uredospores. More recently he (10) reported evidence against the volatile nature of this self-inhibitor.

Experiments were conducted to study the distribution of rust inhibitors in rusted bean leaf tissue. One series of experiments was suggested by the observation that rusted bean leaves were commonly not reinfected, especially when the number of pustules per unit area of leaf surface was high.

Germination inhibitors were detected with the agar diffusion bioassay in rings of tissue adjacent to rust pustules in both bean leaves and wheat leaves. Rings of tissue surrounding a rust pustule were taken at various distances from the center of the pustule and subjected to this bioassay. Sufficient concentrations of germination inhibitors were present in bean or wheat tissue 5–8 mm from the center of the pustule to give 100% inhibition of the uredospore germination of either bean rust or wheat rust. Inhibitors were not detected in tissue more distant from pustules, in stem tissue, or in young non-rusted trifoliate leaves from bean plants with rusted primary leaves.

On a rusted bean leaf, reinfection occurred closer than 5 mm from the center of the pustule when the leaf was incubated in a cool, moist atmosphere. Three groups of bean plants with different concentrations of pustules per unit leaf area were held 24 hours at 3 temperature ranges (10–13°, 24–28°, and 30–35°C) and then inoculated with an aqueous suspension of uredospores. These plants were incubated at 16°C, which was near the optimum temperature for infection to take place, and then moved to a greenhouse chamber maintained at 27°C. After about 7 days, pustules due to the initial infection were clearly distinguishable from those due to reinfection. Ten areas, 1 cm², were assessed for both initial and reinfection pustules.

At the medium and high temperature ranges the number of reinfections was inversely proportional to the number of initial infections, up to 8 initial infections per cm² (Fig. 3). At the low temperature range this relationship was not observed.

The relation of 3 different combinations of incubation and subsequent development temperatures to the inhibition of reinfection was determined. Reinfection was inhibited when temperatures of both incubation and development were 26.5°C and when temperature

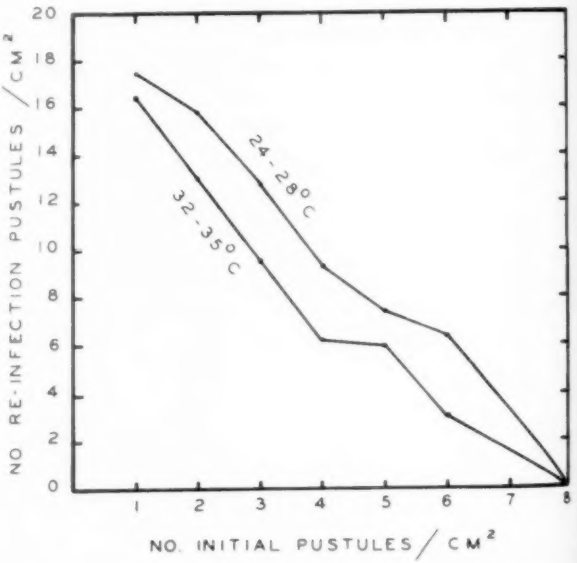

FIG. 3. Protection, due to initial rust infection on primary Pinto bean leaves, against reinfection with the same rust was greater at a medium (24–28°C) than at a high (32–35°C) temperature range.

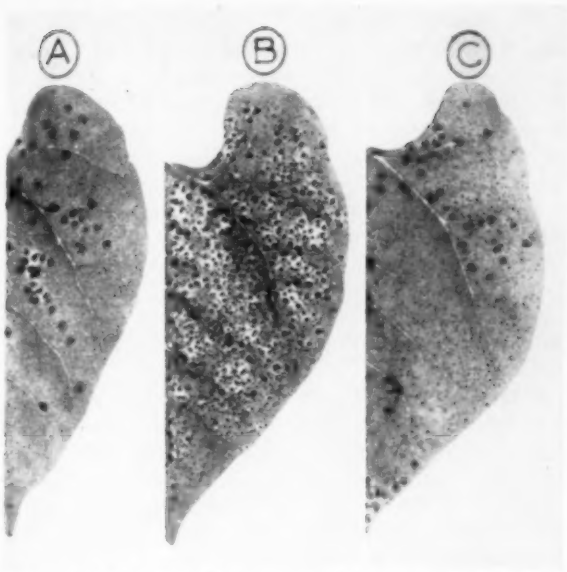

FIG. 4. The effect of temperature on protection due to rust infection. Protection occurred when the temperature of incubation and development was 26.5°C and when the temperature of incubation was 26.5°C and that of development was 16.5°C (A and C), but not when the temperature of incubation was 16.5°C and that of development was 26.5°C (B). Note: the large dark spots were due to initial infection and the small spots were due to reinfection.

of incubation was 26.5°C and temperature of development was 16.5°C, but not when temperature of incubation was 16.5°C and that of development was 26.5°C (Fig. 4). The effect of temperature on bean rust reinfection apparently occurred during the incubation of the uredospore. Whether the self-inhibitors were less active or were decreased in concentration because of more free water on the surfaces of the leaves at 16°C than at 26.5°C was not determined.

Growth regulators neutralize spore self-inhibitors.—Allen (1) reported that the self-inhibitor of uredospores of *Puccinia graminis* var. *tritici* was neutralized by 2,4-dinitrophenol (DNP), by 2-methyl-1,4-naphthoquinone, and by coumarin.

Aqueous solutions of each of these materials and of gibberellic acid were sprayed onto attached rusted bean leaves. After the leaf surface had dried it was uniformly inoculated with a heavy suspension of bean rust uredospores. The plants were placed in a moist chamber at 22°C overnight, and then on the greenhouse bench.

Coumarin at 100 ppm increased the number of reinfections 3-fold, and DNP at 9.2 ppm increased the number of reinfections 4.5-fold. The increase in number of reinfections with coumarin was greater at 100 ppm than at 50 ppm. Neither indole-3-acetic acid at a concentration of 10 ppm nor gibberellic acid at 100 or 1000 ppm increased the number of reinfections.

The effect of these 4 materials on the self-inhibition phenomenon was studied by the use of the agar-diffusion bioassay. The material was added to water agar that had been cooled to 45°C. Pustules, and rings of adjacent tissue around pustules, were cut from rusted bean leaves and assayed for self-inhibitors with substrata containing different concentrations of the material.

On pustules on water agar the germination of bean rust uredospores was inhibited, and only a small percentage germinated on a ring of the tissue surrounding the pustule. The inhibitors from pustules and from adjacent tissue were neutralized by 50 ppm coumarin in the water agar. Gibberellic acid at 100 or 1000 ppm, DNP at 0.29, 0.57, 1.15, 2.3, 4.6, or 9.2 ppm, and indoleacetic acid at 0.312, 0.625, 1.25, 2.5, or 5.0 ppm failed to neutralize the action of the inhibitors from pustules and adjacent tissue.

Self-inhibitors in other rust fungi.—Self-inhibitors of germination have been reported by Allen (1) in *P. graminis* f. sp. *tritici*, by LeRoux and Dickson in *P. sorghi* (6), and by Yarwood (10) in *U. phaseoli* var. *typica*, *P. helianthi*, and *P. antirrhini*.

Self-inhibition of germination was found in *P. polygoni-amphibii* var. *persicariae*, *P. graminis* f. sp. *tritici*, *P. helianthi*, *P. carthami*, and *U. phaseoli* var. *typica*. In the agar diffusion bioassay the inhibitors of any one of these rusts inhibited the germination of any of the others, and also *Cronartium ribicola*. No inhibitors were found in the uredospores of *C. ribicola*.

Self-inhibition independent of photo-inhibition.—Preliminary observations on the influence of light on germination of uredospores of bean rust indicated that as little as 100 ft-c of daylight inhibited spore germination. Both inhibitor-depleted and untreated uredospores of bean rust from the same uredospore harvest were placed in daylight and darkness to compare their sensitivity to light under conditions otherwise favorable to germination. The surface of 10 ml water agar in closed Petri dishes served as substratum for germination. Darkness was provided by wrapping the dishes in aluminum foil. All experiments were conducted under room conditions, and daylight through a glass window was the source of light. Light intensity varied from 50 to 130 ft-c.

The per cent germination of the inhibitor-depleted uredospores was 50% greater in darkness than in daylight, and the germination of untreated uredospores was 70% greater. These results with inhibitor-depleted spores indicated that the self-inhibition phenomenon apparently is independent of the photo-inhibition phenomenon.

Inhibitor-depleted uredospores vs. untreated uredospores in storage experiment.—Inhibitor-depleted uredospores and untreated uredospores from the same bean rust spore harvest were stored in capped vials in the dark at temperatures from −17°C to +31°C. At different time intervals spores were dusted sparingly onto the surface of a 2% water agar in Petri dishes and incubated 20 hours in the dark at 21°C, after which per cent of germination was determined.

These tests showed that, when stored at a relatively high temperature, inhibitor-depleted uredospores remained viable longer than did untreated uredospores.

Inhibitors in sterilization-killed uredospores.—Sterilization-killed uredospores of the bean rust fungus gave a positive test for inhibitors when tested with the agar diffusion bioassay. These uredospores had been treated with propylene oxide vapor (0.2 ml per 500 mg of spores) in closed vials. They did not germinate under favorable conditions of germination after such a treatment, and were assumed to be dead and sterile since there was no evidence of bacterial or fungal growth after 5 days on the surface of potato-dextrose agar medium or potato-dextrose-peptone agar medium.

Discussion.—On the basis of qualitative and quantitative evidence, aspartic and glutamic acids appeared to be the self-inhibitors of bean rust uredospore germination that are present in the aqueous diffusate from uredospores. Whether self-inhibition of uredospore germination through high concentration of spores per unit area of substratum is due to these 2 amino acids alone remained in question. Assuming that each gram of uredospores contained 1 mg of these 2 amino acids and that the average fresh weight per spore was 3.8×10^{-9} g (10), the amount of these acids in the agar was determined. At a spore concentration of $10^7/cm^2$, which gave complete self-inhibition of germination on water agar substratum (9 ml water agar in 9-cm Petri dishes) the substratum contained about 27 ppm of these amino acids. With the pure acids, this concentration did not completely inhibit germination (Fig. 2). However, it was logical to assume that there was formed a concentration gradient of inhibitors around each spore, with highest concentration near the spore itself. Based on a spore density of 1.36 (10) the concentration of self-inhibitors in the uredospore on this agar substratum was about 136 ppm, or more than sufficient to give 100% self-inhibition.

Whether the self-inhibitors were produced by the fungus or by the infected host tissue was not determined. The fact that the uredospores carry self-inhibitors and that the tissue of a pustule also contains inhibitors of spore germination does not enable one to determine the origin of the self-inhibitors. That the self-inhibitors may be readily eluted from the spore with water and are not present thereafter in the spore (inhibitor-depleted uredospores) suggests their presence as a liquid matrix surrounding the spore during its ontogeny. The fact that rusted tissue, before pustules break and uredospores are exposed, does not contain inhibitors of uredospore germination (unpublished data) may support the above suggestion. There is no evidence that the uredospores can produce self-inhibitors; on the contrary, storage experiments with inhibitor-depleted uredospores show that the self-inhibitors are not regenerated on or in such spores.

The distribution of the inhibitors of uredospore germination in the tissues of rust pustules and in the tissues adjacent to the pustules has been indicated. Whether these inhibitors are on the surface of the tissue or between or inside the cells was not determined. The fact that a brief washing of rusted leaves reduced resistance to infection by the same rust (7) is evidence that these inhibitors are on or near the surface of the tissue. Yarwood (10) reported that soaking rusted bean leaves 8 hours in water completely eliminated resistance resulting from rust infection. Wilson (7) reported that resistance in similar tissue was reduced by washing the tissue 3 minutes under the tap.

Whether the inhibitors found in rusted tissue are the same as those found in uredospores of the rust fungus remains undetermined. However, their solubility in water, their neutralization by coumarin, their specificity of activity against the germination of uredospores, and their close association suggest that they are the same.

The infrequent occurrence of germination and the increased germ tube length of bean rust uredospores on a thickly seeded water agar substratum has not been explained in terms of self-inhibitors.

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GENETICS OF RESISTANCE OF THE BARLEY VARIETIES RICARDO AND MODIA TO SEVERAL CULTURES OF ERYSIPHE GRAMINIS F. SP. HORDEI[1]

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SUMMARY

The inheritance of resistance to various cultures of races of *Erysiphe graminis* f. sp. *hordei* was studied in the Ricardo (C.I. 6306[3]) and Modia (C.I. 2483) barley varieties. Ricardo has 1 dominant gene conditioning resistance to cultures BC3, T4, NC9, and VA11. Modia has 1 dominant gene conditioning resistance to cultures BC3, CR3, T4, NC9, VA11, and CAN12, and 1 additional dominant gene conditioning resistance to cultures BC3, CR3, and T4. The reactions of 145 F_2 lines from the cross Modia x Kentucky 1 to inoculation with cultures CR3 and CAN12 demonstrated that resistance to both cultures was conditioned by the same dominant gene, and that the second dominant gene conditioning resistance to culture CR3 was inherited independently.

INTRODUCTION.—The genetics of resistance of barley to powdery mildew, *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal, has been studied for many years. The purity of the host species of *Hordeum* or varieties of *Hordeum vulgare* was maintained in the early studies, but the purity of the pathogen was disregarded. Since 1930, when races of the pathogen were identified by Mains and Dietz (10), specific races have been used in most studies of inheritance of this resistance. It has been realized in recent years that the type of infection is the result of an interaction of genes in the host and the pathogen. This has led to studies of host-parasite interactions.

The inheritance of resistance in barley varieties to specific races of powdery mildew has been studied extensively, but only a few investigators have studied simultaneous inheritance of resistance to more than 1 race. In this study the inheritance of resistance was determined in the varieties Ricardo (C.I. 6306) and Modia (C.I. 2483) to cultures of 6 races of powdery mildew.

REVIEW OF LITERATURE.—Many investigators have reported results from studies on the inheritance of resistance in barley to powdery mildew. Because several publications on this subject were reviewed by Smith (17) in 1951, only the information since made available is discussed here.

Schaller and Briggs (16) designated the gene for resistance to California race 3 in the Black Russian variety as $M1_{a2}$. They found this gene to be allelic to the $M1_a$ gene in the Algerian variety. Five genes ($M1_k$, $M1_a$, $M1_{a2}$, $M1_d$, and $M1_p$) of the 10 they identified are in linkage group II.

Hiura and associates (6, 7, 8) isolated 11 genes in barley that condition resistance to several races of powdery mildew in Japan, and designated them $M1_1$ through $M1_{11}$. They showed that, in certain varieties, 1 gene conditioned resistance to some races, but 2 or 3 genes conditioned resistance to other races. The relationship of the genes in some varieties was found to be similar to that reported by the California workers. Since different pathogenic strains of the pathogen were used, the genes identified in these 2 studies cannot be accurately compared.

Favret (2, 3, 4) isolated, as genetic testers, 6 genes (A, C, M, P, Z, and GO) that condition resistance to race A-2 in Argentina. He also identified 5 mutants that condition resistance to this race in the progeny from seed irradiated with gamma-rays or neutrons. The relationship between the 5 mutant genes induced by irradiation and the 6 genes used as genetic testers is being determined.

The inheritance of resistance in the variety Bolron (C.I. 7123) to race 3 from British Columbia was investigated by Atkinson (1). Although distinct genes for resistance were not isolated, it was concluded that this variety has 2 or 3 genes that condition resistance to that race.

Huntley (9) found that the varieties Psaknon, Stephan, Sel. 175, and Kwan each possessed 1 dominant gene that conditioned resistance to his culture of race 9 of powdery mildew, whereas the Duplex variety had 2 dominant genes. The gene in the Stephan variety had a different locus from those in the other 4 varieties.

MATERIALS AND METHODS.—The barley varieties used in this study were Kentucky 1 (C.I. 6050), Smooth Awn 86 (C.I. 6269), Modia (C.I. 2483), and Ricardo (C.I. 6306). The first 2 are susceptible to most of the pathogenic strains of powdery mildew in North America. The other 2 have been resistant, in the seedling stage, to all pathogenic strains of the organism in North America with which they have been inoculated (11, 13, 14); they were resistant in the field at all locations where the Uniform Barley Mildew Nursery has been grown in the United States and Canada (12).

The crosses were made in the greenhouse at Blacksburg, Virginia, and the F_1 plants grown at Aberdeen, Idaho. The F_2 seeds were space-planted in the field

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[3] C. I. refers to the accession number of the Cereal Crops Research Branch, Crops Research Division.

TABLE 1.—*Description and origin of the 6 cultures of Erysiphe graminis f. sp. hordei*

Stock culture No.	Physiologic race[a]	Originated from a single	From plants grown at
BC3	3	Ascospore	Agassiz, B. C., Canada
CR3	3	Conidia	Davis, California
T4	4	Ascospore	Beeville, Texas
NC9	9	Conidia	Statesville, N. Carolina
VA11	11	Ascospore	Blacksburg, Virginia
CAN12[b]	12	Ascospore	Winnipeg, Man., Canada

[a] The physiologic race of each culture was determined by the reaction of the 6 differentiating varieties: Black Hulless (C.I. 666), Chevron (C.I. 1111), Goldfoil (C.I. 928), Heil's Hanna (C.I. 682), Nepal (C.I. 595), and Peruvian (C.I. 935), as described by Newton and Cherewick (15).

[b] Stock culture number CAN12 is the same as culture 12A1 used in previous studies.

at Warsaw, Virginia, and the F_3 lines used in this study were taken at random.

Each of the 6 cultures of powdery mildew originated from a single conidia or a single ascospore. In Table 1 the cultures are described as to physiologic race, culture number, type of spore derived from, and where original material collected. The cultures were classified as to physiologic race by the reaction of the 6 differentiating varieties, as described by Newton and Cherewick (15). The cultures will be referred to by their stock culture number.[4]

Inoculations were made in a greenhouse at about 70°F or in a growing room at 66–69°F with 550 ft-c of light from white fluorescent tubes for 16 hours a day. Unless indicated otherwise, at least 15 plants of each F_3 line were inoculated with each culture. Reactions were essentially the same in greenhouse and growing room. Plants were inoculated in the first-leaf stage and readings were made 8–10 days later. Reactions of plants to infection were recorded by a method similar to that published by Moseman (14). The general description of reaction types is as follows: 0 = immune; 1 = highly resistant; 2 = moderately resistant; 3 = moderately susceptible; and 4 = very susceptible.

[4] Stock culture number CAN12 is the same as culture 12A1 used in previous studies.

RESULTS.—Table 2 shows how parent varieties, F_1 plants, and F_3 lines taken at random from the crosses Ricardo × Smooth Awn 86 and Modia × Kentucky 1 reacted to inoculation with various races of powdery mildew. The frequency of homozygous resistant, heterozygous, and homozygous susceptible F_3 lines from the Ricardo × Smooth Awn 86 cross inoculated with cultures BC3, T4, NC9, and VA11 indicates that 1 gene conditions resistance in Ricardo. It conditions resistance to all these races, since each of the 118 F_3 lines reacted the same to the 4 cultures. This gene is completely dominant since the F_1 plants of this cross were as resistant as the resistant parent, and in the F_3 lines that were segregating for resistance there was a 3:1 ratio of resistant to susceptible plants.

The 145 F_3 lines from the Modia × Kentucky 1 cross reacted differently to cultures NC9, VA11, and CAN12 than to cultures BC3, CR3, and T4. The frequency of F_3 lines homozygous resistant, heterozygous, and homozygous susceptible to the former cultures indicates that 1 gene conditions resistance to them. The frequency—7 homozygous resistant to 8 heterozygous to 1 homozygous susceptible—of F_3 lines

TABLE 2.—*Seedling reaction[a] of parent, F_1 plants, and F_3 lines of 2 crosses to inoculation with various cultures of Erysiphe graminis hordei*

Cross and culture tested	First parent listed	Second parent listed	F_1 plants	Number of F_3 lines			Value of P for ratio indicated
				R	Seg.	S	
Ricardo × Smooth Awn 86							
BC3	R(0–1)	S(4)	R(1)				
T4	R(1)	S(4)	R(1)				
NC9	R(1)	S(4)	R(1)	32	60	26	
VA11	R(1)	S(4)	R(1)				
Lines expected (1:2:1)				29.5	59	29.5	.80–.70
Modia × Kentucky 1							
NC9	R(1)	S(4)	R(1)				
VA11	R(0–1)	S(4)	R(1)	29	73	43	
CAN12	R(1)	S(4)	R(1)				
Lines expected (1:2:1)				36.25	72.5	36.25	.20–.10
Modia × Kentucky 1							
BC3	R(1)	S(4)	R(1)				
CR3	R(1)	S(4)	R(1)	60	72	13	
T4	R(1)	S(4)	R(1)				
Lines expected (7:8:1)				63.5	72.5	9	.50–.30

[a] The reactions were read on a 0–4 scale: 0–1 = immune to highly resistant, 1 = highly resistant, 4 = very susceptible. R denotes resistant, S susceptible, and Seg. segregating.

TABLE 3.—*Seedling reaction of parents and F_3 lines from the cross Modia × Kentucky 1 to inoculation with cultures CR3 and CAN12 of Erysiphe graminis hordei* R(1)

Culture tested	Parent and genotype		Postulated genotype of F_2 plants and reaction of F_3 lines to indicated culture							Value of P for ratio indicated
	Modia	Ky.1								
Postulated genotype	AABB	aabb	AABB AABb AAbb	AaBB	aaBB	Aabb	aaBb	AaBb	aabb	
CR3	R(1)	S(4)	R	R	R	Seg.3:1	Seg.3:1	Seg.15:1	S	
CAN12	R(1)	S(4)	R	Seg.3:1	S	Seg.3:1	S	Seg. 3:1	S	
Lines observed			29	23	8	22	22	28	13	
Lines expected* (4:2:1:2:2:4:1)			36.25	18.1	9.1	18.1	18.1	36.25	9.1	.30–.20

* Number of lines of each genotype expected if one of the dominant genes in Modia conditions resistance to both culture CR3 and culture CAN12, while the second, independently inherited, dominant gene conditions resistance only to CR3.

to the second 3 cultures indicates that 2 independent genes condition resistance to these races. The resistant reaction of the F_1 plants and of plants in the segregating populations indicates that these 3 genes are dominant.

Table 3 shows the reaction of each of the 145 F_3 lines from the Modia × Kentucky 1 cross to cultures CAN12 and CR3. The tests included at least 20 seedlings from each F_3 line that was susceptible to both races, and at least 50 seedlings from lines that were resistant or segregating for resistance to either race.

The probability value from the chi square for goodness of fit to the 4:2:1:2:2:4:1 ratio indicates that one of the 2 genes conditioning resistance to culture CR3 is the same as the gene conditioning resistance to culture CAN12, and that the second gene is inherited independently.

DISCUSSION AND CONCLUSIONS.—One dominant gene in Ricardo conditions resistance to cultures BC3, T4, NC9, and VA11 of powdery mildew. One dominant gene in Modia conditions resistance to cultures BC3, CR3, T4, NC9, VA11, and CAN12, while a second, independently inherited, dominant gene conditions resistance only to cultures BC3, CR3, and T4. The method of utilizing a variety resistant to several pathogenic cultures of a pathogen in a breeding program will depend on the number of genes conditioning resistance to these cultures. Breeding for resistance is simplified when the resistance of a variety to several cultures is conditioned by a single gene, since tests with any one of the cultures will ascertain whether this resistance has been transferred. If the resistance of a variety to various cultures is conditioned by different genes, then it is necessary to test the breeding progenies with a series of cultures, each of which is pathogenic on all but 1 of the genes that condition resistance in the host, in order to determine when the complete resistance of the variety has been transferred. In developing multigenic lines, a variety that has more than 1 gene conditioning resistance would be a desirable parent provided tests can be made for the presence of each gene.

The results from this study illustrate the importance of testing the same progeny lines from a cross with pathogenically different cultures of the pathogen to determine the relationship of genes conditioning resistance to these cultures. Such tests with F_3 lines from the Modia × Kentucky 1 cross showed that one of the 2 independently inherited genes conditioning resistance to cultures BC3, CR3, and T4 also conditioned resistance to cultures NC9, VA11, and CAN12.

Any tests of the resistance in Modia to additional pathogenic strains of powdery mildew will necessitate the determination of the identity of the gene or genes involved with the 2 known genes. This could be accomplished by comparing the reactions of selected F_3 lines from this study to the new culture and to cultures CR3 and CAN12. The relationship of an unknown gene to the 2 known genes also could be determined by comparing the reactions of the F_3 lines when inoculated with culture CR3 and the new culture.

In comparing the gene or genes in Modia that condition resistance to a new culture of powdery mildew with the 2 known genes, it would be necessary to use the cultures of the races from this study, since 2 cultures of the same race may differ in their pathogenicity on varieties other than the differentials. Atkinson found that the Kwan variety was resistant to a culture of race 3 from California, but susceptible to a culture of race 3 from British Columbia. This also has been found true for cultures CR3 and BC3, by the senior author.

If the relationship that Flor (5) described between the genes for virulence in flax rust, *Melampsora lini* (Pers.) Lev., and those that condition resistance in host flax, *Linum usitatissimum* L., applies to powdery mildew, *E. graminis* f. sp. *hordei*, and its host barley, *Hordeum vulgare* L., then cultures NC9, VA11, and CAN12 have 1 gene in common for virulence on Modia that is not present in cultures BC3, CR3, or T4.

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SOME FACTORS AFFECTING PENETRATION AND INFECTION BY SCLEROTINIA SCLEROTIORUM[1]

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SUMMARY

Investigations were made of the influence of several factors on the formation of appressoria by germinating ascospores of different isolates of *Sclerotinia sclerotiorum*. Results indicated that a carbon source was essential for formation of appressoria, though appressoria formed in solutions lacking other essential elements when carbon was available. Mechanical contact also was necessary for formation of appressoria: these structures formed in the suspending solution only in contact with a cover slip. Spermatia-producing branches were formed at random in all test solutions, and their formation was not influenced by the presence or absence of any one essential element. Ascospores of different isolates infected different host plants when applied to leaves in suspending solutions provided with a carbon source. No infections resulted when the suspending solution lacked a carbon source, unless the inoculation was made at the cite of natural or artificial wounds. Freshly ejected ascospores of clover isolates penetrated directly and infected host leaves in 3–4 days, except for tomato leaves, which were infected only after 10–11 days. Ascospores of lettuce and tomato isolates infected only partially senescent bean leaves. Mycelium originating from sclerotia was infective only through an intermediary of non-living organic matter. Histological studies showed that infection by ejected ascospores of clover isolates took place 36 hours after the formation of a single appressorium at the tip of the germ tube. Mycelium was observed in epidermal cells after 60 hours. Disorganization of cellular contents of guard cells prior to penetration was observed when ejected ascospores of clover isolates germinated 1–2 cells away from the stomatal opening. There were no instances of disorganization of epidermal cells beneath germinating ascospores.

Several reports have compared the importance of ascospores and sclerotia in the penetration and infection of susceptible hosts by *Sclerotinia sclerotiorum* (Lib.) d By. emend. Purdy (15). Some workers have reported that germinating ascospores of the fungus penetrated directly through epidermal cell walls (1, 5, 6, 11, 12, 21); others have indicated that infection by ascospores takes place only through the intermediary of non-living organic matter (13, 16, 19).

Some reports (10, 12) have indicated that sclerotia function in perpetuating the fungus only by their ability to produce the perfect stage, and that the mycelium originating from sclerotia is non-infective; others indicate that soil-borne sclerotia are the principal sources of inoculum in perpetuation of the organism (4, 8, 14, 22, 23).

According to Brown (3), an appressorium or modification thereof must be formed for mechanical penetration to occur. Boyle (2) described the formation of appressoria by ascospores germinating in a nutrient solution, and indicated that penetration was accomplished by mechanical pressure. He reported no evidence of injury to the host before penetration. DeBary (7) reported that 2 conditions were necessary for the formation of appressoria: 1) ascospores must germinate in water, not in a nutrient solution; and 2) mechanical contact must be made between the fungus and some solid object. He also reported that penetration of the host was made possible by prepenetration killing of host tissue. Several workers (18, 20, 22) have reported that unwounded healthy host tissues were infected by ascospores applied in a nutrient solution, but not by ascospores suspended in water. The present work was undertaken to resolve some of these conflicts and to obtain additional information on the penetration and infection processes of *S. sclerotiorum*.

EXPERIMENTAL RESULTS.—*Formation of appressoria.*—Since appressoria are prerequisite to penetration (3), some of the factors that influence their formation were investigated. Ascospores of isolates from lettuce, ladino clover, or tomato were germinated in distilled water, complete nutrient solutions, nutrient solutions lacking C or N, or solutions lacking essential mineral elements (K, P, Mg, Fe, Cu, Zn, Mn) but containing a carbon source. Both complete and incomplete solutions were prepared as described by Purdy and Grogan (17). Ascospores from the different isolates were suspended in the different nutrient solutions, placed on cover slips, and inverted over van Tiegham cells. Four van Tiegham cells were placed on moistened filter paper in each Petri plate and incubated at 18°C. Each test was replicated 8 times, and experiments were repeated 3–5 times.

Ascospores germinated in distilled water or in solutions lacking a carbon source, but did not form appressoria, regardless of the presence or absence of mineral elements essential for prolonged growth.

Appressorial masses were formed by ascospores that germinated in 5% solutions of dextrose, sucrose, galactose, levulose, lactose, or soluble starch. Soluble starch apparently contained some soluble carbohydrate that was utilized by the fungus, for there was

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Grateful acknowledgment is made to Professor R. G. Grogan, under whose supervision this work was done.

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no indication that the starch grains themselves were utilized.

Appressorial masses did not form at random in the solutions suspending the spores, but only in contact with the cover slip. Upon contact with the cover slip, the germ tubes branched dichotomously (Fig. 1, A–D). One of the 2 branches occasionally continued vegetative growth, but, more commonly, both branches formed a hand-like structure, and the terminal cells became enlarged at the tips. The circular areas of these terminal cells were the only points of contact between the fungus and the cover slip. Eventually, a small, pore-like structure appeared in the circular area of each terminal cell. Its formation has been described by Boyle (2), and interpreted as the initiation of a penetration peg. This structure did not become thread-like, but protruded only slightly at the tip of each branch. The appressorial masses were darker in color than the supporting hyphae, which were hyaline.

These results indicate that the principal factors governing the formation of appressoria are the presence of a carbon source and the stimulus of mechanical contact.

Formation of spermodochia.—The formation and development of spermatia-producing branches could possibly be confused with appressorial development (Fig. 1, E–G). However, spermatia-producing branches on the hyphae were more commonly initiated some distance from the terminal cells, and were formed in solutions lacking a carbon source as well as in all other solutions. A protuberance was formed that was perpendicular to the long axis of a hyphal cell. It increased in size until the typical bottle-shaped spermatiophore was produced, from which spermatia were formed and abstricted. Spermatiophores usually branched repeatedly, forming a compact spermodochium with spermatia formed terminally by all branches. More rarely, a spermatiophore was initiated by a terminal dichotomy, with one branch continuing vegetative growth and the other branching freely to form a spermodochium. Appressorial masses differed from spermodochia by being formed only in contact with a mechanical barrier, the cover slip, whereas spermodochia were produced at any locus along a hyphal filament. Appressorial cells were larger and darker in color than the smaller hyaline cells of spermodochia.

Penetration of host tissues by germinating ascospores in liquid suspension.—Detached leaves of red clover, ladino clover, lettuce, broccoli, Brussels sprouts, bean, and tomato were inoculated with ascospores of the several isolates suspended in the different nutrient solutions. The inoculated leaves were

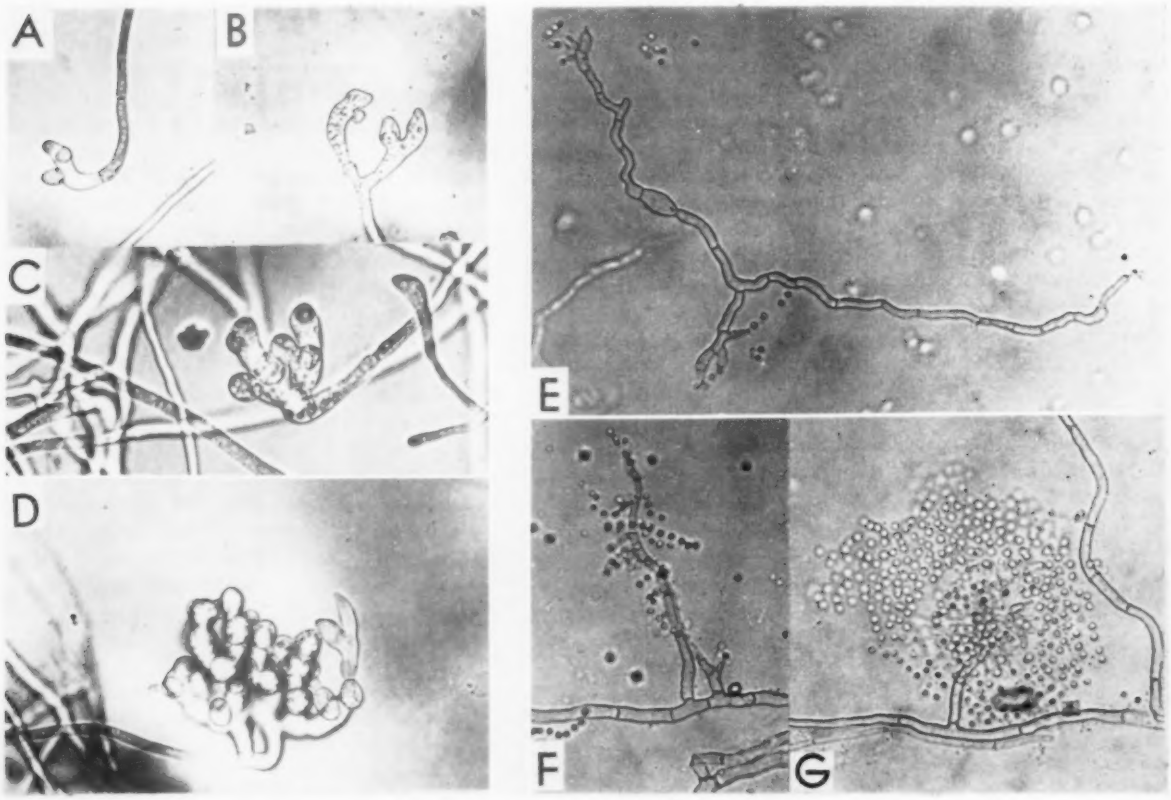

FIG. 1. A–D) Stages in the development of appressorial masses in hanging-drop preparations. E–G) Development of spermatia-bearing branches; G) Mature spermodochium. ×400.

placed in glass-covered dishes, either on wire screen platforms or directly on the surface of sterile distilled water. Three dishes were used for each nutrient solution and fungus isolate, and each dish contained 1–3 leaves of the host being inoculated. The dishes were incubated at 12°C, and each experiment was repeated 3 times.

Regardless of host or isolate, infection failed when inoculum consisted of ascospores suspended in distilled water, except for leaves of Brussels sprouts inoculated at the loci of natural or artificial wounds. However, all leaves became infected when inoculated with ascospores suspended in solutions containing a carbon source.

Observations in fields of Brussels sprouts heavily infected with *S. sclerotiorum* indicated that nearly all infections took place where rifts had occurred along the leaf margins, or at points on the leaves that had been previously infected by *Mycosphaerella brassicola* (Fr. ex Duby) Lindan. No infection appeared to have originated in healthy intact tissue.

Infection by germinating ascospores ejected from apothecia.—Leaves of lettuce, broccoli, Brussels sprouts, ladino clover, red clover, broad bean, bean, and tomato were inoculated with ascospores of several different isolates from lettuce, ladino clover, red clover, and tomato. To suspend apothecia of the different isolates above leaves of the various hosts, the base of the apothecial stipe was pressed into a block of water agar on the underside of the cover of a glass-covered dish. One to 4 apothecia were placed in each dish, and the same number of leaves were floated on a small volume of sterile distilled water or supported on wire screens above the water. The dishes were incubated at 12°C. Three dishes were used for each host and fungus isolate, and all experiments were repeated 3 times.

In almost every instance, leaves were infected in 3–4 days by freshly ejected ascospores of the ladino clover and red clover isolates, as evidenced by discoloration of the epidermal cells. Tomato leaves inoculated with ascospores of these same isolates showed signs of infection only after 10–11 days. Leaves detached this long had probably become senescent, and therefore more susceptible to infection.

Inoculations with ascospores from the lettuce and tomato isolates infected only bean leaves, and then only if they were partially senescent. Germination of the ascospores took place, but there was no evidence of the formation of appressoria.

Infection by mycelium from sclerotia.—The importance of sclerotia in disseminating and perpetuating the diseases caused by *S. sclerotiorum* has been subject to controversy. Keay (10) and Loveless (12) reported that sclerotia function only to produce the perfect stage, not directly serving as inocula. Several other workers have reported that sclerotia are the main source of inocula and the principal means by which the fungus is disseminated (4, 8, 14, 22, 23).

Mycelium from sclerotia of all isolates produced appressoria when germination took place in sterile distilled water. However, healthy unwounded hosts did not become infected when inoculated with sclerotia that germinated in distilled water. Infection occurred only when sclerotia were placed near wounds or in a nutrient solution.

In a lighted 12°C room, sclerotia produced by lettuce isolates were placed on the soil surface close to lettuce plants from which all dead leaves had been removed. No infection resulted until after 2 months, when 1 plant was infected. The fungus gained entry through a dead leaf attached to the plant.

In other experiments in the greenhouse, lettuce plants with dead leaves removed, 4 rows per flat, were inoculated with sclerotia of lettuce and ladino clover isolates, placed ½ in. from the base of the plants. In 2 of the rows, non-living lettuce leaves placed over the sclerotia were allowed to contact the base of the plants. In 2 weeks, all these plants were dead or infected; in the rows without dead-leaf contact the plants were not infected, although the sclerotia had germinated and mycelium had contacted the crowns. Results were similar when sclerotia of other isolates were used as inoculum.

Lettuce plants in the field usually remain free from infection until after the rosette stage. Then the lower leaves become senescent or die, becoming an avenue of entry for the fungus. Infection originates from sclerotia in soil, which serve as inocula and are the principal means by which the fungus is perpetuated.

Histological studies of inoculated leaves.—Detached leaves of red clover, ladino clover, lettuce (var. Bibb), Brussels sprouts, broccoli, bean, and broad bean were inoculated with ascospores of isolates from ladino clover and lettuce, in distilled water or complete nutrient solutions; or with ascospores ejected from apothecia. For 5 days after inoculations, small pieces of inoculated tissue were cut from leaves at 6-hour intervals. Tissue pieces were fixed and killed in a modified Karpechenko's solution (9), washed in running water, and dehydrated in a standard ethyl-alcohol–butyl-alcohol series, embedded in tissuemat, sectioned at 7–10 μ, and stained for 24 hours in 0.25% phloxine followed by 20–30 seconds in 0.25% fast green. The relationship of ascospores to points of penetration was also observed in inoculated leaves cleared in lactophenol and stained with cotton blue. Leaves inoculated with ascospores in a nutrient solution were penetrated initially by a single appressorium formed at the tip of the germ tube followed by the formation of appressorial masses (Fig. 2, A–B), in nearly all cases without the formation of an obvious subcuticular vesicle. However, a subcuticular vesicle was formed in penetrations of lettuce associated with appressorial masses (Fig. 2, A). Host cells immediately beneath the point of penetration collapsed, followed by disorganization of the remaining tissue, as evidenced by the yellowish-brown staining properties of the affected cells.

Paraffin sections and cleared leaves inoculated with

ascospores in distilled water showed that germination had occurred, but appressoria had not formed and penetration was not accomplished.

Penetration took place in 36 hours in leaves inoculated with ascospores ejected from apothecia of the clover isolates. A single appressorium formed at the tip of the germ tube (Fig. 2, C–D), and the hyphae were seen in epidermal cells beneath the appressorium after 60 hours. The germ tube was generally short, and the appressoria were always formed before penetration.

Killing or injury of tissues before penetration, the theory proposed by DeBary (7), was observed occasionally when ascospores germinated near stomata. Leaves of broccoli, red clover, and ladino clover inoculated with ejected ascospores from clover isolates showed disorganization of the contents of guard cells when ascospores germinated 1–2 cells away from the stomatal opening, even though there was no evidence of penetration. The affected guard cells stained a yellowish-brown, in contrast to the green- and red-staining affinity of unaffected cells. Many instances of affected guard cells were observed without evidence of mycelium within the stomatal opening or in epidermal or sub-epidermal cells. The germinating ascospores apparently produced a diffusible substance that entered the stomatal opening and disorganized the cellular contents of guard cells before actual penetration of the host. However, this toxic substance was apparently incapable of transcuticular diffusion, for there was no evidence of cellular disorganization beneath germinating ascospores that were not associated with stomata. In suspending liquids this substance is apparently too dilute to be effective.

Discussion.—Although evidence presented supports the prepenetration-killing theory proposed by DeBary (7), penetration was more commonly accomplished directly through the cuticle and epidermis beneath an appressorium when inoculations were made with ejected ascospores, whereas ascospores suspended in a liquid initiated infections only if a carbon source was available. It can be concluded that the nutrients necessary for the formation of appressoria by germinating ascospores as well as the substance capable of prepenetration killing are apparently diluted by pure water to ineffectiveness. Undamaged host tissue inoculated with sclerotia developed infection only when some form of non-living organic matter was present or when the sclerotia were germinated in a solution containing a carbon source.

It is apparent that sclerotia function both as a direct source of inoculum and also in their capacity to

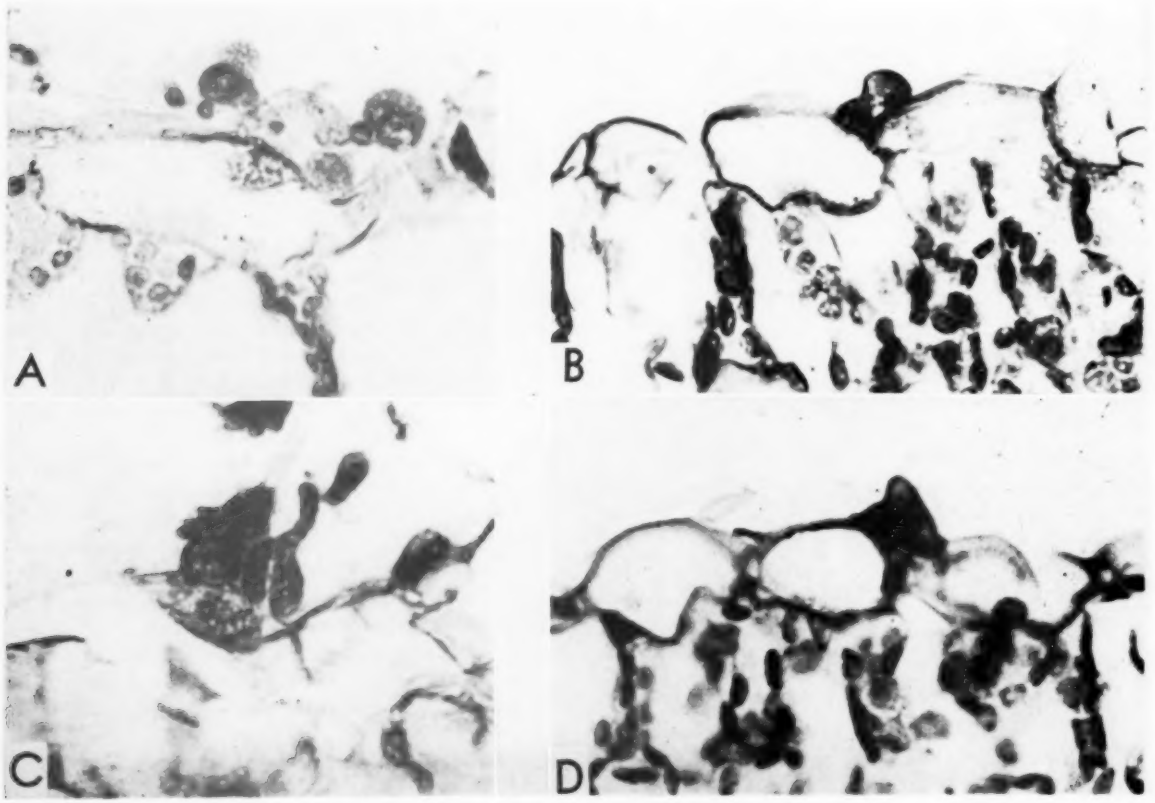

Fig. 2. A–B) Penetration of Bibb lettuce by ascospores germinating in a nutrient solution. A) Formation of subcuticular vesicles; B) without a subcuticular vesicle. C–D) Germinating ascospores with appressoria attached to epidermal cells. ×600.

produce the perfect, or ascospore, stage. These 2 phases vary in importance with the host and with variations in host susceptibility in different stages of development.

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VERTICILLIUM WILT OF GARDEN STOCK (MATTHIOLA INCANA)[1]

Robert D. Raabe and Stephen Wilhelm[2]

SUMMARY

Verticillium wilt of garden stock, *Matthiola incana*, has been found prevalent in cut-flower-producing regions of northern and southern California. The symptoms, including a yellowing and wilting of the lower foliage, are very similar to those of potassium deficiency, and in some ways resemble those of Fusarium wilt, Rhizoctonia foot rot, bacterial blight, and Phytophthora foot rot.

Inoculations have shown the susceptibility of such common types as the column type, branching type, and ten weeks type, and of the evening-scented stock. In 2 inoculation series, the column-type stock was found susceptible to isolates of *Verticillium albo-atrum* from stock, tomato, radish, and a nightshade weed.

The first report of Verticillium wilt of stock, *Matthiola incana* R. Br., was in 1938, by Oyler (10). In studying the host range of an isolate of *Verticillium albo-atrum* Reink. & Berth. from chrysanthemum, she noted that stock was one of the susceptible plants. In 1940, Smith (12) reported the natural occurrence of the disease in California. Cunningham, in 1949 (6), reported stock as being susceptible to *V. dahliae* in New Zealand. In 1950, Snyder, Hansen, and Wilhelm (13) reported occurrence of the disease in a home garden in California, noting that the symptoms differed somewhat from those reported by Smith. They also indicated the possibility of strain differences in the pathogen since, according to a New York report (7), stock did not become infected when inoculated with an isolate from chrysanthemum. Baker and Sciaroni (2) reported in 1952 that Verticillium wilt of stocks occurs infrequently in California.

The symptoms of Verticillium wilt of stocks appear as a progressive yellowing and wilting of the leaves, beginning at the base of the plant (Fig. 1, 2). On individual leaves, the yellowing begins at the margins and progresses inward, sometimes following the veins so that the interveinal tissues are the last to lose their color (Fig. 3). Plants infected early in their development may be stunted severely. The vascular tissues of infected plants are frequently discolored, the discoloration extending up through the petioles into the leaves (Fig. 2B).

There is an apparent similarity between the symptoms of stock verticilliosis and those of the Fusarium wilt of stock (1). They differ somewhat in that the lower leaves of plants infected with Verticillium do not abscise as much as those infected with Fusarium. They also differ in that there is not the tendency for unilateral development or for the tawny coloration and flattening of seed pods that characterize the Fusarium wilt disease.

Other parasitic diseases of garden stocks that produce symptoms similar to those of Verticillium wilt are Rhizoctonia foot rot, bacterial blight, and Phytophthora foot rot. *Rhizoctonia solani* Kühn causes a yellowing and wilting of the lower leaves by girdling the plants at the soil level (8). The affected tissues turn black and become markedly constricted. These lesions are often restricted to one side of the stem at first, leading to one-sided development of the plant. Bacterial blight, caused by *Xanthomonas incanae* (Kend. & Baker) Starr et al., can be differentiated by the characteristic blackening of the leaf scars (9). Phytophthora foot rot, resulting from infection by *Phytophthora cryptogea* Peth. & Laff. (14), produces a rapid wilting and drooping of most of the leaves. The lower part of the stem collapses in a dark, soft, wet decay.

The external symptoms of Verticillium wilt of stock are also similar to those resulting from potassium deficiency, as pictured by Cook and Millar (5, Fig. 39). Post also pictured this deficiency (11, p. 637) and stated that stocks are very sensitive to a lack of potassium. Cahoon and Crummett (3, 4) reported the deficiency from field plantings in California and studied the potassium nutrition of stocks. Much of the yellowing of stock plants in the cut-flower-growing area in the San Francisco Bay region was believed to result from this deficiency. The isolation of Verticillium from a stock plant in a home garden prompted a survey of the flower fields in the Bay area and of the seed fields near Santa Maria, California. Plants showing symptoms were selected at random and cultured. *V. albo-atrum* was found in plants from many of the stock fields from the Bay area, but not in plants from

[1] Accepted for publication June 18, 1958.

[2] Assistant Professor of Plant Pathology and Associate Professor of Plant Pathology, respectively.

FIG. 1–3. Fig. 1. Verticillium wilt in inoculated seedling stocks, showing degrees of stunting and stages of wilting and yellowing of lower leaves. Uninoculated check plant is on the left.—Fig. 2. Verticillium wilt in inoculated stock just before bloom. Vascular tissues of leaves exposed by cutting off a portion of the midrib. A) Uninoculated check plant and portion of leaf showing exposed vascular tissue. B) Inoculated plant showing collapsed lower leaves and portion of a leaf showing dark coloration of vascular tissues.—Fig. 3. Leaves from infected plants showing various stages of yellowing and wilting. Leaf at left is healthy.

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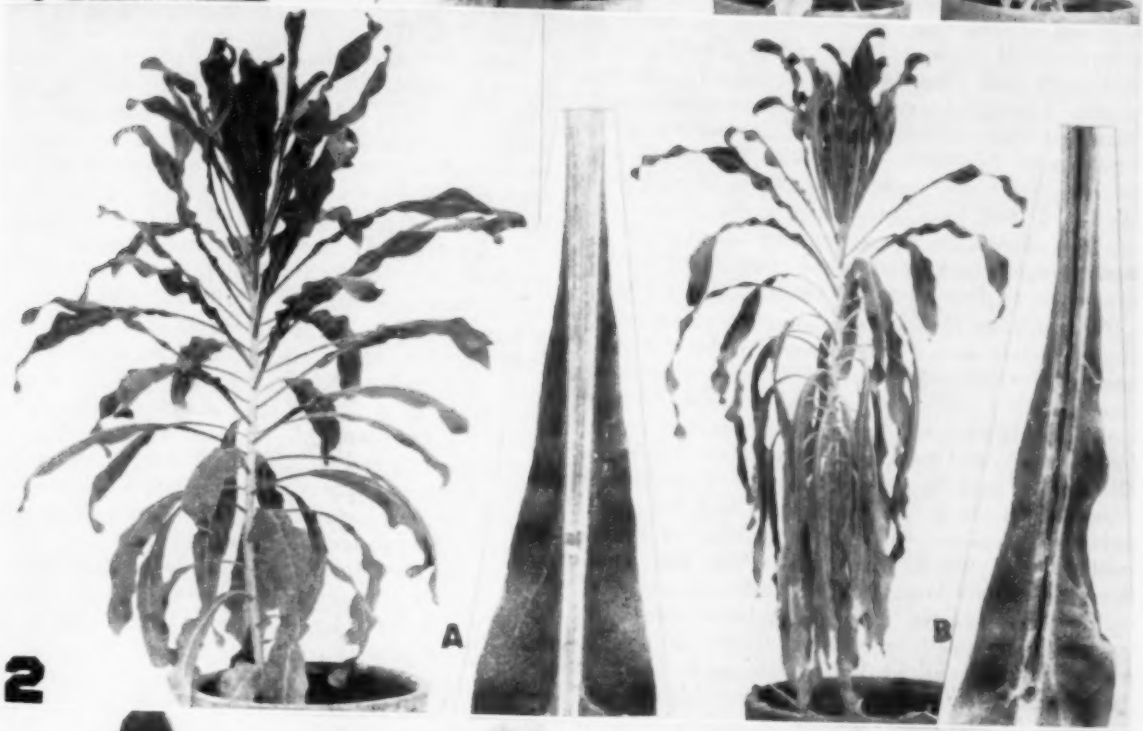

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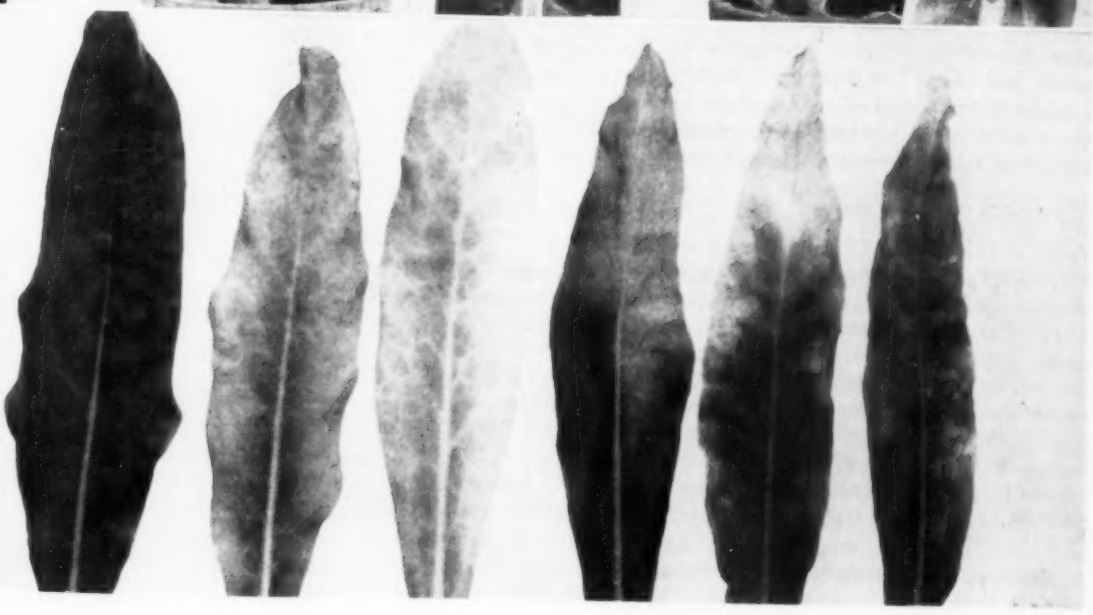

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the Santa Maria area. Following this, a report from A. O. Paulus[3] revealed the disease to be prevalent in several stock-growing areas of southern California.

The widespread distribution of Verticillium wilt in stock indicated the need for further study of the disease. To determine the susceptibility or resistance of the different types of stock, seedlings of each were inoculated by washing the roots and dipping them in a spore suspension of a culture of the fungus isolated from stock. The types used included the following varieties of *M. incana:* Double Giant Excelsior (column type), Tall Giant Imperial, Perpetual Giant Perfection, Trisomic Giant Imperial (branching types), Beauty of Nice; one variety of *M. incana* var. *annua* Voss, Dwarf Ten-Weeks stock; and *M. bicornis* DC, the evening-scented stock. Five plants of each were inoculated, and in about 2 months the plants were cultured. Typical wilt symptoms developed in all the inoculated plants, and, upon culturing, *V. albo-atrum* was recovered from them but not from the checks.

To determine if isolates from other plants would attack stock, a second experiment involving 2 isolates from the nightshade weed (*Solanum sarachoides* Sendt.) and 1 each from radish (*Raphanus sativus* L.), Brussels sprouts (*Brassica oleracea* var. *gemmifera* Zenker), and tomato (*Lycopersicum esculentum* Mill.) were used in inoculation tests. Two different isolates from the garden stock were included in the tests. Eight plants each of 3 varieties of column stocks (Giant White, Light Shell Pink, and Bright Rose Pink) were inoculated in 2 different ways with each of the 7 isolates. In one inoculation series, seed was sown into soil infested by pouring a 100-ml suspension of the desired isolate into each 6-in. pot. After germination, the seedlings were thinned to 1 per pot. In the other inoculation series, seedlings were dipped in a spore suspension of the desired isolate and planted in 6-in. pots. Each series included 4 uninoculated plants of each variety, as checks, making a total of 360 plants in the test. All soil was fumigated with chloropicrin before use.

[3] Personal correspondence.

The surviving plants were cultured about 4½ months after inoculation. Five pieces from each plant were plated on natural media plates. The results (Table 1) show that stock is quite susceptible to all the isolates used except the one from Brussels sprouts. This isolate grows extremely slowly in culture and, perhaps for this reason, is slow to infect plants.

The frequent inability to infect all plants of a susceptible species upon inoculation with Verticillium, as here with stocks, has been encountered in other studies (15, 17).

The symptoms of the inoculated plants that became infected varied from mild (slight chlorosis of lower leaves) to severe (plants extremely stunted and leaves small and strap-like). With all isolates, symptoms were more severe on the variety Bright Rose Pink than on the other varieties. A number of the plants died during the experiment and were not counted in the final tally. It is assumed that many died as a result of infection by the fungus.

Discussion.—Verticillium wilt of stock, formerly not recognized as an important disease, has recently been found to be widely scattered in the San Francisco Bay region and in southern California, where garden stocks are grown for cut flowers. The similarity of the symptoms of this disease to several other diseases of stock has made field diagnosis very difficult. Accurate diagnosis, based on laboratory culturing of tissues of suspected plants, is important since control measures for the diseases differ.

The apparent freedom of the seed-producing areas in California from Verticillium wilt of stocks, together with the general lack of seed transmission of the Verticillium diseases, points to the soil as a reservoir of the pathogen.

In control of Verticillium wilt of stocks, consideration must be given to the following factors: 1) the finding that stock is susceptible not only to Verticillium isolates from other crucifers but also to isolates from tomato and the nightshade weed, 2) the widespread occurrence of Verticillium in the floricultural crop land areas, and 3) the evidence that the fungus remains alive in soil for long periods (16). Fumiga-

Table 1.—*Ratio of infected plants to plants surviving inoculation with V. albo-atrum*

Variety of stock	Isolate from: Nightshade weed #124	Nightshade weed #N-1	Stock #134	Stock #105	Radish #53	Brussels sprouts #115	Tomato #9	Check
Soil inoculated [a]								
Giant White	3/7	1/7	3/7	0/8	2/7	0/7	3/7	0/3
Light Shell Pink	3/7	3/8	3/8	4/6	5/7	0/8	1/8	0/3
Bright Rose Pink	3/7	3/7	4/6	4/7	2/5	0/8	0/7	0/3
Seedling inoculated [b]								
Giant White	3/7	3/8	3/5	3/8	2/6	0/7	6/7	0/3
Light Shell Pink	7/8	4/6	0/8	1/8	0/7	2/8	4/8	0/4
Bright Rose Pink	5/8	4/8	1/7	2/8	4/8	1/8	8/8	0/3
Total	24/44	18/44	14/41	14/45	15/40	3/46	22/45	0/19

[a] Soil was infested with a spore suspension.
[b] Seedlings were dipped in a spore suspension before being planted.

tion with chloropicrin has been shown to be successful in ridding infected soils of the pathogen (18).

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THERMAL DEATH RANGE OF VERTICILLIUM ALBO-ATRUM[1]

Paul E. Nelson[2] and Stephen Wilhelm

SUMMARY

Minimum exposure and lethal temperature in hot water for a rose and a tomato isolate of *Verticillium albo-atrum* growing on previously sterilized uniform pieces of flower stalks of *Plantago lanceolata* was 5 minutes at 47°C for hyphae and conidia, and 10 minutes at 50°C for microsclerotia. A 40-minute exposure at 47°C also killed microsclerotia. Rose and pelargonium cuttings known to be infected with *V. albo-atrum* failed to root after treatment in hot water at several temperatures lethal to *V. albo-atrum*. Microsclerotia exposed in dry atmosphere at 49-50°C survived 6 months. Conidia in such an atmosphere succumbed within 3 days. Microsclerotia of 2 other isolates survived 2½ years at 40°C.

In the fall of 1950, experiments were started to determine the effect of hot-water treatments on *Verticillium albo-atrum* Reinke & Berth. The principal objective was to determine if the hot-water temperature lethal to the fungus was safe for plant materials such as cuttings, budwood, and nursery stock so that the fungus could be eliminated from them. A preliminary report has already appeared (10).

REVIEW OF LITERATURE.—Many plant diseases are perpetuated and spread by methods of vegetative propagation used by nurserymen and growers alike. Heat treatments have been used as a means of control of some of these diseases. Baker and Cummings (2) found that Pythium root rot of *Aloe variegata* L. could be controlled by immersing infected plants 30 minutes in hot water at 46°C. Baker and Durbin (3) reported that Pythium root rot of *Haworthia attenuata* Haw. was controlled with the same temperature and time. Baker and Chandler (1) reported that hot-water treatment can be used to obtain pathogen-free propagating material of such plants as *Dieffenbachia picta* Schott, *Fittonia verschaffeltii* Coem. var. *argyroneura* Nichols, *Pellionia pulchra* N. E. Br., *Syngonium auritum* (L.) Schott, and *S. podophyllum* Schott var. Emerald Gem. Dimock and Baker (5) found that Phytophthora root rot of calla (*Zantedeschia* sp.) could be controlled by treating infected rhizomes 1 hour in hot water at 50°C. The effectiveness of heat and hot-water treatments against certain virus infections in propagative materials is well known (7, 8). Other examples of control of pathogens carried in propagating stock by heat treatment can be found in the literature.

Various workers (4, 11, 14) have demonstrated that budwood taken from rose plants infected with *V. albo-atrum* may transmit the disease in budding to

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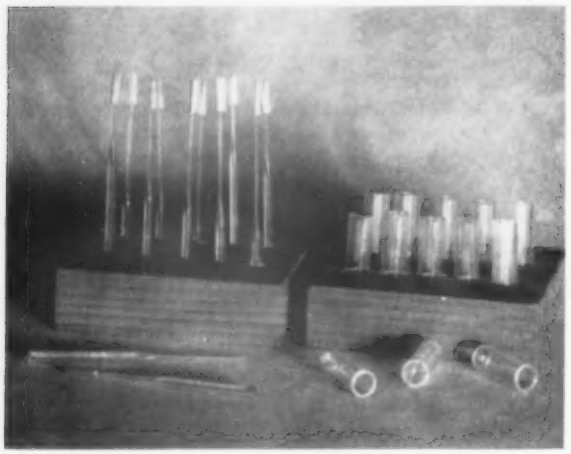

FIG. 1. Glass needles and vials used in the hot-water treatments—in place in wooden blocks. Note the pieces of Plantago flower stalk on the tips of the glass needles.

canes of susceptible varieties. Dimock (4) reported that buds taken from Verticillium-infected rose canes and budded into healthy susceptible varieties, either on their own roots or on Manetti understock, established infection above the inserted, infected bud in 35% of the plants budded. Infection was confirmed by isolation of the fungus from the cane above the infected bud. Raabe and Wilhelm (11) reported that a high percentage of bud failures and diseased plants resulted when buds of Red Delight and Red Garnet rose varieties were inserted with a Verticillium-contaminated budding knife. Brushing a spore suspension on an uninfected lopping wound 1 month after budding yielded a lower percentage of infected plants. A low percentage of infected (conspicuously stunted) plants resulted when buds were taken from naturally infected wood. On the basis of these reports it was felt that hot-water treatment of rose cuttings and budwood might be of some value in controlling Verticillium wilt in roses.

MATERIALS AND METHODS.—Two isolates of *V. albo-atrum*, one from tomato and another from rose, were used in this study. All cultures used were initiated from single spores.

Flower stalks of *Plantago lanceolata* L. were selected to grow the fungus on because they are hollow and have a uniform diameter throughout much of their length, and because the fungus sporulates well on them and forms abundant microsclerotia in and on them. Flower stalks, cut into pieces about 1 cm long, were sterilized with propylene oxide (6) before use. After a few tests, autoclaving 20 minutes at 15 lb. was found to moisten the pieces and make them more suitable for colonization by the fungus.

Pieces of autoclaved plantago stems were dipped in a suspension of spores of *V. albo-atrum* and incubated in Petri dishes on 2% water agar for varying periods, depending on the stage of fungus desired for treatment. If hyphae, conidia, and microsclerotia were desired, incubation was for 7 days. If the treatment was to be on hyphae and conidia only, incubation was for 36–48 hours.

The temperature of constantly stirred water in a glass tank of about 18-liter capacity was held at ±0.5°C of treatment temperature by adding boiling water through a funnel as needed. During each test the water temperature was checked every 2 minutes.

Ten stalk pieces were placed on the tips of 10 glass needles about 7 cm long that were held securely about 1 cm apart in a small wooden block (Fig. 1). Inverted blocks floated in the treatment tank for the desired time held the fungus inoculum about 12 cm deep. After treatment the stalk pieces, still on the needles held by the inverted block, were cooled by immersing them in 10 separate vials of cold sterile water held in place in a second block of wood (Fig. 1). This arrangement eliminated the chance of contaminating a dead inoculum piece with one possibly still containing live Verticillium.

The cooled pieces were touched to a sterile blotter to remove excess moisture and plated on barley-straw-water agar (12). After 1 week the first reading was taken, on the basis of hyphae and conidia of the fungus present. After 3 weeks the final reading was taken, on the basis of microsclerotia in the pieces of straw in the medium.

RESULTS.—*Hot-water treatments.*—The first series of tests was conducted to determine the time and temperature required to kill hyphae and conidia of *V. albo-atrum*. The results of these tests, on both isolates of the fungus, are summarized in Fig. 2A. A minimum of 5 minutes at 47°C was required to kill mycelium and conidia of the fungus.

The second series of tests was conducted to determine the time and temperature required to kill microsclerotia of the fungus. There was considerable variation in treatment results, partly because microsclerotia are more difficult to kill: the escape of a single microsclerotium in an air bubble or otherwise would give a positive reading even though all other microsclerotia were killed. The results of these tests are summarized in the graph in Fig. 2B. A minumum of 10 minutes at 50°C was required to kill microsclerotia of the fungus. A 40-minute exposure killed microsclerotia at all temperatures.

The third series of hot-water treatments was carried out on plant material. Cuttings of pelargonium (var. Grand Slam) and rose (var. Ragged Robin and Better Times) were used in these tests. All cuttings were first culture-indexed to determine whether they were infected with *V. albo-atrum*. Only infected cuttings were used.

The pelargonium cuttings were divided into 2 groups, one consisting of soft, herbaceous cuttings and the other of hard, woody cuttings. Cuttings from each group were used in each treatment. The treatments were for 5 minutes at 55°C, 10 and 30 minutes at 52°C, 20 minutes at 50°C, and 60 minutes at 45°C. After treatment the cuttings were placed, for rooting,

in 2½-in. pots in a half-sand and half-soil mixture. All cuttings died without rooting, indicating that times and temperatures required to kill *V. albo-atrum* were too severe for treating pelargonium cuttings infected with this fungus.

Rose cuttings (var. Ragged Robin) were treated 30 minutes at 52°C, 10 minutes at 51°C, and 60 minutes at 45°C. Reculturing the treated cuttings showed that all treated at 52° and 51°C were free of the fungus, as were 9 of 10 treated at 45°C. No attempt was made to root these treated cuttings.

Rose cuttings (var. Better Times) were treated for 10, 20, and 30 minutes at 52°, the same periods at 50°C, 30 minutes at 48°C, and 90 minutes at 45°C. Half the treated cuttings were placed in cold storage at about 3°C, and the rest were placed, for rooting, in pots in a half-sand and half-vermiculite mixture. After 1 week the other cuttings were removed from cold storage to pots. All cuttings died without rooting, indicating that times and temperatures required to kill *V. albo-atrum* are too severe for the treatment of rose cuttings infected with this fungus.

Dry-heat treatments.—To test the effect of dry heat on conidia of *V. albo-atrum*, pieces of Plantago flower stalk were dipped in spore suspensions of each isolate, placed in sterile Petri dishes, and incubated at 49, 46, 43, 40, 37, and 34°C. Culturing showed that conidia were killed in 3 days at 49°. They survived longer at the lower temperatures, but at the end of 7 days were all dead.

To test microsclerotia of *V. albo-atrum*, pieces of Plantago flower stalk were dipped in spore suspensions of each isolate and incubated 15 days on 2% water agar at room temperature. The pieces were then placed in sterile Petri dishes and allowed to dry at room temperature for 14 days. They were then incubated at 49, 46, 43, 40, 37, and 34°C. At 1, 2, 4, and 7 weeks, and thereafter every 2 weeks until the 35th week, when the experiment was terminated, 3 pieces with each isolate were removed from each temperature treatment and cultured on barley-straw–water agar. The plates were read at 1 and 3 weeks; the final reading was based on the presence of microsclerotia in the straw pieces of the medium around the pieces of inoculum.

The results indicated that, at 6 months, a consistent kill of microsclerotia of both isolates was obtained at 49, 46, and 43°C. By 8 months a consistent kill was obtained at 40°C, whereas no consistent kill was obtained at 37 and 34°C at any time during the experiment. Microsclerotia of 2 other isolates of *V. albo-atrum* survived 2½ years at 40°C.

DISCUSSION.—The results of the hot-water treatments on *V. albo-atrum* differ somewhat from those reported by Miller and Stoddard (9), possibly because they used a different isolate of the fungus and treated the fungus while it was growing on agar in test tubes. That is a more artificial technique than was used in the tests reported herein; the authors feel it is less accurate and more difficult to relate to the treatment of living plant material. However, Miller and Stoddard (9) also concluded that hot-water treatment would be of doubtful value in freeing dormant strawberry runners of *V. albo-atrum*.

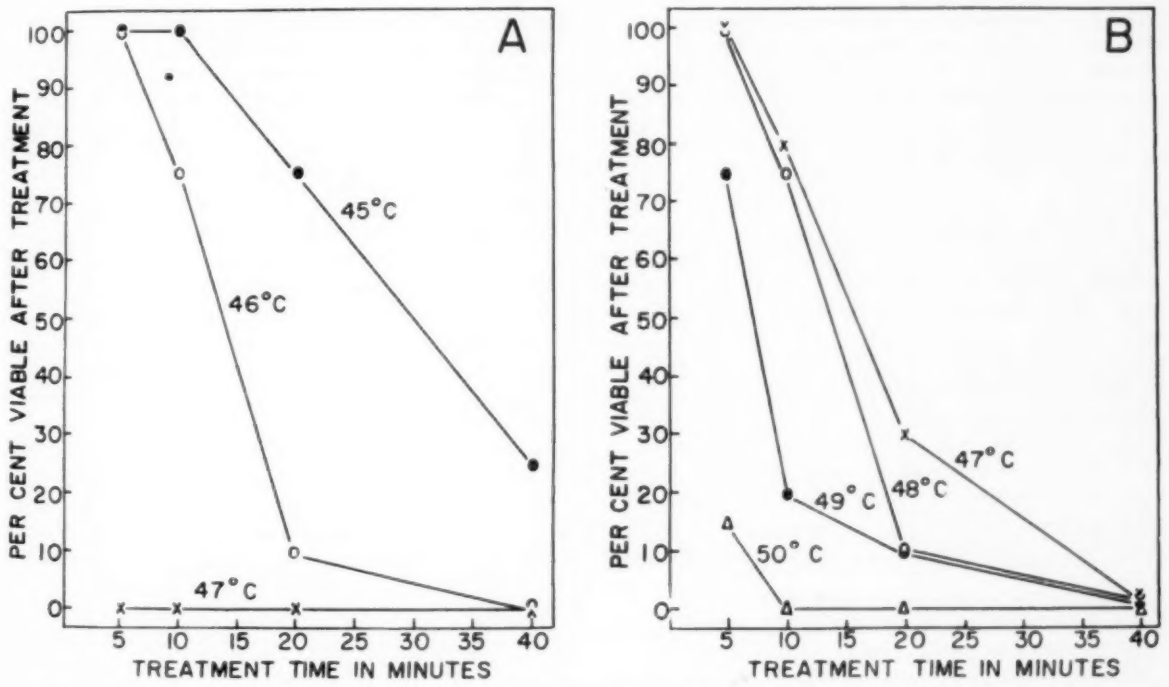

FIG. 2. The effects of hot-water treatments on A) hyphae and conidia, and B) microsclerotia of *Verticillium albo-atrum*.

The ability of the microsclerotia to survive for long periods, as shown in the dry-heat experiments, is undoubtedly important in the carry-over of *V. albo-atrum* from year to year in infested soil in semi-arid regions. Wilhelm (13) reported that, particularly in the central and southern parts of California, *V. albo-atrum* is able to persist several years in soil cropped continuously with non-susceptible grain crops. In one particular instance this persistence extended over 8 years.

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DECAY OF FIBROUS ROOTS OF CITRUS[1]

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SUMMARY

The importance of *Phytophthora citrophthora* and *P. parasitica* as destroyers of fibrous feeder roots of citrus was demonstrated. Environmental factors favoring the parasitism are excess water and organic matter in the soil. Seedlings fertilized with ammonium sulfate or urea were more susceptible to attacks by these fungi than those receiving calcium nitrate. Trifoliate orange rootlets showed resistance to parasitism by these fungi. Control of fibrous root rot in susceptible rootstocks can be effected by using uncontaminated or disinfected seed; planting in disinfested seedbed, nursery, and orchard sites; irrigating with disinfested water; protecting planting sites during first 2 years from contamination by run-off water; and practicing the best cultural methods.

Citrus trees in poor condition generally have few functioning feeder roots. Examination reveals either a small number of short, stubby roots or sloughing roots whose decaying bark easily slips off the stele under slight pressure. Destruction of small feeder roots may be caused primarily by parasitic fungi or by starvation resulting from girdling by rodents, gummosis fungi, tristeza virus, or other biological factors, or by direct injury from herbicides, spray materials, or fertilizers so carelessly applied that harmful concentrations remain in contact with the bark. The main purpose of this paper, however, is to discuss the *Phytophthora* spp. known to parasitize the small roots, and the relationship of this parasitism to several cultural and environmental factors, and to suggest methods for controlling the trouble.

Fraser (5, 6), Klotz (7, 8, 9, 10), Sokoloff and Klotz (16), Klotz and Sokoloff (13, 14), Klotz, DeWolfe, and Wong (12), and Klotz and DeWolfe (11) pointed out the role of soil microorganisms in the destruction of roots, and described soil conditions favoring fungal attack and the relationship to toxic chemicals. While nitrite nitrogen in an acid medium is injurious to roots of citrus and avocado, Bingham and Zentmyer (2) and Zentmyer and Bingham (18) could not demonstrate that the chemical accelerates root rot by *Phytophthora* spp. In an experiment in Florida, Ford, Reuther, and Smith (4) found that young Valencia orange trees with Rough lemon rootstocks given large applications of ammonium nitrate (2.4–3.5 lb. per tree per year) had at the end of a 6-year period 60% fewer feeder roots by weight than

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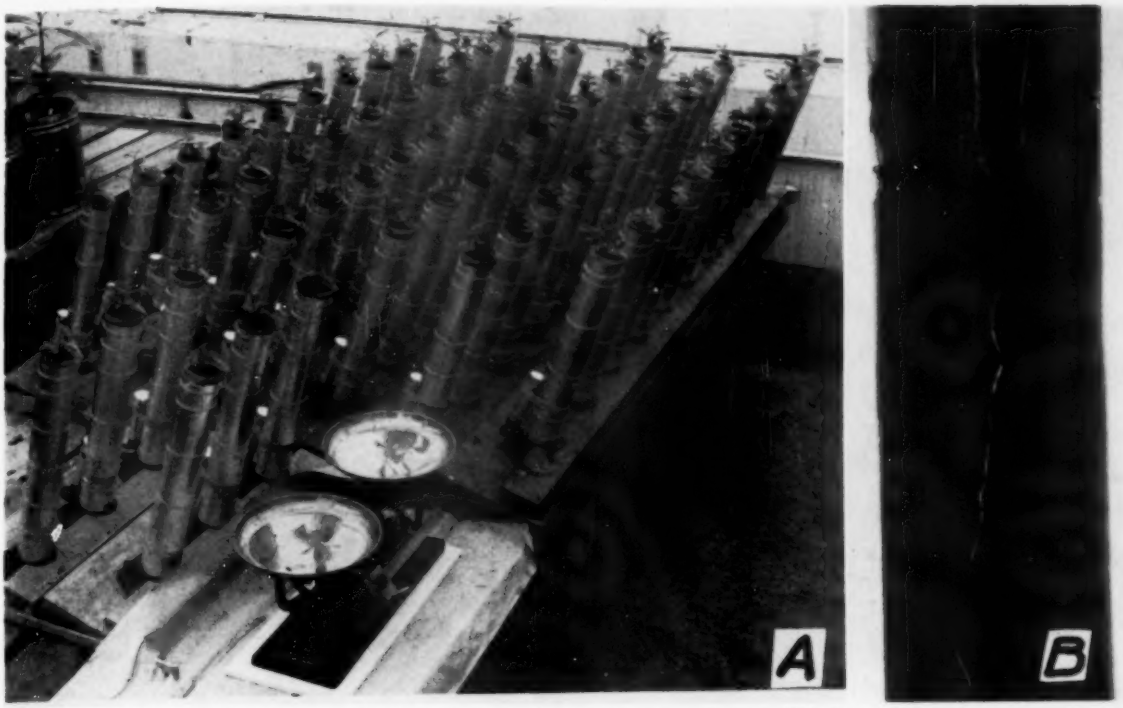

FIG. 1. A) Equipment for study of effect of soil moisture, organic matter, and kind of inorganic nitrogen on Phytophthora root rot of sweet orange seedlings; B) aluminum foil removed from cylinder for observation of roots.

those given .6–.87 lb. (low N), and 13.2% fewer than those receiving 1.2–1.75 lb. (medium N). Martin et al. (15), using sweet orange seedlings in fumigated soil in 3-gal. earthenware pots, showed that, of the several fungi added to the cultures, only *Thielaviopsis basicola* (Berk. & Br.) Ferr. caused marked reduction in growth. This is remarkable in that no evidence, either from field specimens or from inoculation of seedlings in pure culture, has yet been secured of parasitism of citrus roots by this fungus, although Klotz identified *Thielaviopsis basicola* (Berk. & Br.) Ferr. growing on citrus roots in the solution cultures of Chapman and Brown (3). These latter two investigators, moreover, pointed out that high phosphate concentrations favored infection by *T. basicola* of rootlets of sour orange in solution culture, and that acidifying the solution to pH 3.5 checked the growth of the fungus and the infection on newly formed rootlets. So far we have been unable to infect citrus rootlets in sand culture with this fungus, although, as indicated above, *T. basicola* can cause lesions on the small roots growing in nutrient solutions. The results of Martin et al. may have been due to parasitism of the roots by the fungus and/or to a toxic environment that the fungus provided under the conditions of the experiment. This fungus has been isolated only infrequently from roots of orchard trees but commonly from orchard soils.

EXPERIMENTAL RESULTS.—*Pathogenicity of Phytophthora spp.*—Although *Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leonian and *P. parasitica* Dast. have been found to be active parasites of citrus rootlets, these fungi can also develop with great rapidity as saprophytes on roots killed by waterlogging, by products of fermentation (8), by overdoses of fertilizer chemicals, or by other factors. Thus, a build-up of the population of these fungi may be promoted by certain adverse soil conditions. The following experiment demonstrates that these 2 fungi can be active parasites under soil moisture conditions unfavorable to the host. Sweet orange seedlings were grown in a disinfested soil in tall (44-cm) glass cylinders of 1-liter capacity. By means of a small glass cylinder connected with a rubber tube to an opening near the base of the larger glass culture cylinder, a water table was maintained at the 10-cm level above the bottom of the soil of some of the cultures (Fig. 1). Table 1 shows the percentage of roots destroyed in the presence and absence of the 2 *Phytophthora* spp. under 2 conditions of soil moisture, 2 conditions of organic matter, and 4 conditions of inorganic nitrogenous fertilization. The percentage of root bark destroyed was estimated after decayed bark was removed by slight pressure and pull between thumb and fingers.

Under the conditions of this experiment the importance of the fungi and the simultaneous presence of excess water and organic matter is evident from the relative number of roots rotted.[3]

In another similar experiment[4] Homosassa sweet

[3] The assistance of L. C. Masters in these experiments is gratefully acknowledged.

[4] In this experiment we were ably assisted by Saburo Hashimoto.

TABLE 1.—*Phytophthora root rot of Homosassa orange seedlings after 82 days[a] as influenced by soil moisture, organic matter, and source of inorganic nitrogen*

	% of roots rotted (6-plant averages)			
	Calcium nitrate[b]	Urea[b]	Ammonium sulfate[b]	No inorganic nitrogen
Phytophthora,[e] water table,[d] organic[c]	23.35	66.67	86.67	38.50
Phytophthora, water table, no organic	3.00	47.50	59.17	8.50
Phytophthora, no water table, organic	0.00	1.01	4.50	0.03
Phytophthora, no water table, no organic	1.17	1.70	0.47	2.51
No Phytophthora, water table, organic	0.00	0.67	0.17	0.00
No Phytophthora, water table, no organic	0.00	0.41	0.00	0.50
No Phytophthora, no water table, organic	0.02	0.03	0.83	0.00
No Phytophthora, no water table, no organic	0.00	0.00	0.00	0.00

[a] Set up March 6, 1953.
[b] 6 lb. N to 400 sq. ft.
[c] 400 lb. horse manure per 20×20-tree square.
[d] Water table at 10-cm level above bottom of 42-cm soil column; started May 29, 1953.
[e] Mixture of *Phytophthora citrophthora* and *P. parasitica* added June 1 and 16, 1953.

orange seedlings were grown in cans (5200-cc capacity) containing 6000 g of soil disinfested with chloropicrin or by drying and autoclaving. However, none of the cultures were deliberately subjected to the conditions accompanying a free water table, and the attempt was made with tensiometers and by weighing to maintain moisture conditions near the optimum (16% of dry weight) for the seedlings.

The amount of root rot caused by the *Phytophthora* spp. was sufficient (Table 2) to retard growth of the plants in the cultures receiving urea, ammonium sulfate, and organic matter. Inoculated seedlings that were supplied with organic matter had the greatest amount of root rot. The smaller amount of root rot in the dried and autoclaved soil is probably due to the destruction of facultative parasites that follow

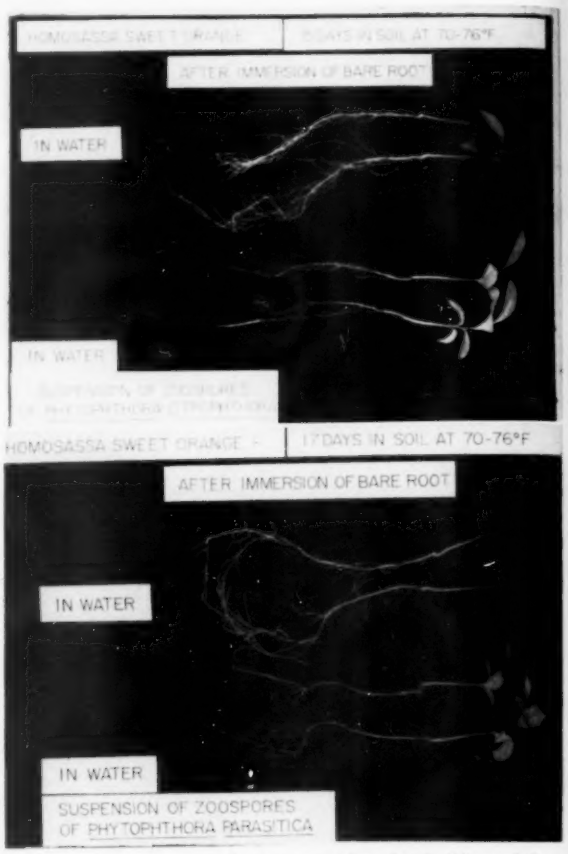

FIG. 2. Destruction of citrus rootlets by 2 *Phytophthora* spp. Note that bark of rootlets of seedlings exposed to the fungi has been destroyed, leaving the woody stele exposed.

invasion by the *Phytophthora* spp. or due to injury from residual effect of chloropicrin.

As shown in the following experiment, most of the root system of some rootstock seedlings in solution or water cultures at 23°C can be destroyed in 10 days by the addition of a zoospore suspension of these fungi to the water (Fig. 2, 3). Four sets of 4 seedlings (12 weeks from seed) of each variety shown in the following tabulation were fastened with rubber bands to the sides of paraffined blocks of wood and

TABLE 2.—*Root rot of Homosassa sweet orange seedlings[a] after 139 days[b] as influenced by Phytophthora spp., organic matter, and source of nitrogen*

	% of roots rotted (3-plant averages)							
	Calcium nitrate		Urea		Ammonium sulfate		No inorganic nitrogen	
	C[d]	H[e]	C[d]	H[e]	C[d]	H[e]	C[d]	H[e]
Phytophthora and organic	0.7	0.5	21.0	14.0	18.0	0.8	18.0	7.6
Phytophthora, no organic	3.7	0.2	28.0	2.4	14.0	0.6	11.0	1.0
No Phytophthora, organic	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]
No Phytophthora, no organic	[c]	[c]	[c]	[c]	0.3	[c]	[c]	[c]

[a] Seed planted December 1, 1953, in cans of 5200-cc capacity with 6000 g of soil.
[b] Inoculation May 6, 1954; harvest, September 22, 1954.
[c] No more than trace of root rot.
[d] Soil disinfested with chloropicrin (C).
[e] Soil disinfested by drying and autoclaving (H).

TABLE 3.—*Relation of certain soil fungi to decay of fibrous roots of citrus*

Inoculum	Root rot	Brown rot of orange
Pythium sp., *Fusarium* sp., and *Mucor* sp.	—[a]	—
Phytophthora citrophthora, *Fusarium* sp., and *Pythium* sp.	+[b]	+
P. citrophthora, *Pythium* sp., and *Mucor* sp.	+	+
P. citrophthora, *Fusarium* sp., and *Mucor* sp.	+	+
Control (sterile soil, water, alfalfa stems)	—	—

[a] —, no rot of roots or indicator orange.
[b] +, rot of roots and/or indicator orange; *P. citrophthora* reisolated.

floated in water so that only the roots were immersed. The seedlings thus mounted were distributed among 4 11-liter battery jars so that each jar had 4 seedlings of each variety. The cultures were aerated by passing compressed air through glass tubes with sintered glass tips extending to the bottom of the battery jars. The fungi were grown on sterile alfalfa stems, which were then enclosed in cheesecloth sacks and suspended in the water near the surface. Zoosporangia and zoospores were thus produced in abundance (Fig. 4C). Fig. 3 shows that the roots of all 7 rootstock seedlings were attacked.

Evidence of the role of *Phytophthora* spp. in root destruction has been secured in the orchard also. A survey of 5 declining and 2 high-producing groves in 5 southern California counties was made to determine the incidence of these fungi. From samples of soil and fibrous roots taken with a 3-in. auger to a depth of 2 ft. around 800 trees in the 5 deteriorating orchards, there were obtained 370 isolates of *Phytophthora* spp.—an incidence of 46%. From 260 trees in the good groves, only 33 cultures of these fungi (13%) were isolated. All samples were taken from the inside shoulder of the first irrigation furrow on the north side and just under the canopy edge of the tree. This is the moist, cool side of the tree, where root rot is generally found. In the drier root zones, nearer the tree trunk, root rot is less common and *Phytophthora* is seldom isolated.

For further observation of the possible relationship of *Phytophthora* spp. to root rot under field conditions, the following experiments were made. By carefully digging so as not to injure and detach them from the tree, masses of healthy fibrous roots were exposed in the drier root zone, near the tree trunks. Porcelain pans containing soil from under the first north-side furrow were placed under these roots. Water was added to the pans of soil and roots, and an inverted pan of the same size was used as a cover. The entire unit was then covered with soil. After 14 days, many of the fibrous roots were rotted. In a similar set of experiments roots were placed in disinfested furrow soil, and *Phytophthora* spp. that had been isolated from soil under the first furrow and grown on alfalfa stems were added to the moistened soil in the pans. An orange fruit was also placed in each pan as a trap to recover the fungi that cause brown rot. As controls, similar units were set up with sterile alfalfa stems. After 2 weeks, none of the controls showed any rotting whereas the roots of the inoculated series showed much rotting and sloughing of the cortex. The roots in the control units remained healthy.

In a third set of similar experiments with sterile greenhouse soil, other species of soil fungi commonly isolated were tested alone and in combination with *P. citrophthora*, as shown in Table 3, which shows that the roots were rotted only when *P. citrophthora* was included.

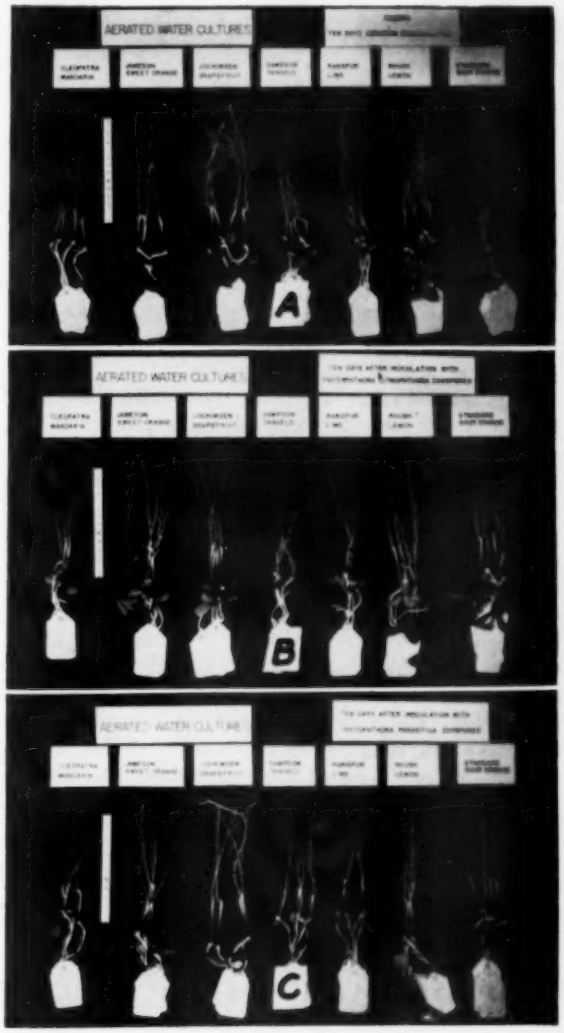

FIG. 3. A) Healthy, uninoculated seedlings of several varieties whose roots were kept 10 days in aerated water; B) like A, but inoculated with zoospores of *Phytophthora citrophthora*; C) like A, but inoculated with zoospores of *P. parasitica*. Note healthy roots of Jochimsen grapefruit, which is resistant to *P. parasitica*.

TABLE 4.—*Percentage decay of fibrous roots by 2 species of Phytophthora in aerated water at 23°C*

	P. citrophthora		*P. parasitica*		*P. citrophthora* + *P. parasitica*
	%	Order of resistance	%	Order of resistance	Order of resistance[a]
Ichang pummelo	80	9	10	3	(7)
Yuzu	70	8	25	5	(8)
Troyer citrange	60	7	50	6	(8)
Rubidoux trifoliate	30	4	10	3	(4)
Texas trifoliate	25	3	10	3	(3)
Frost diploid trifoliate	50	6	20	4	(6)
Frost tetraploid trifoliate	5	1	1	1	(1)
Barnes trifoliate	15	2	5	2	(2)
Pomeroy trifoliate	40	5	10	3	(5)
Webber-Fawcett trifoliate	25	3	10	3	(3)
USDA trifoliate	30	4	10	3	(4)

[a] From mean of % decay by *P. citrophthora* and *P. parasitica*.

Resistance of trifoliate orange to root rot.—Because of the alleged resistance of trifoliate orange, *Poncirus trifoliata* (Linn.) Raf., to root rot (5, 6), several selections of the seedlings of this species and of Troyer citrange, Ichang pummelo, and the Yuzu orange were tested inoculating with zoospores in aerated water in battery jars, as described above.

It is seen (Table 4) that, even under conditions extremely favorable for root rot, the trifoliates showed considerable resistance.

Control measures.—To give the young nursery tree the best opportunity to become established in a new planting, a number of things should be done to protect it from infection by these fungi. From the results of the experiment (Table 5) we learned that seeds for the production of rootstock seedlings can be freed of brown rot infection by 4 minutes of immersion in well-agitated water at 120–125°F. This treatment is especially recommended for seeds from fallen fruit, which are most liable to become infected by brown rot fungi. The treatment does not impair germination and assures the nurseryman that he will not contaminate his seed bed or nursery from this source of infection. The seeds and nursery trees should, of course, be planted in virgin or disinfested soil. Baines et al. (1) eradicated nematodes and *Phytophthora* spp. from roots of young, bare-root sour orange nursery trees by immersing the roots in water at 113°F, 116°F, or 119°F for 25, 10, or 5 minutes, respectively.

TABLE 5.—*Percentage recovery of Phytophthora spp. from heat-treated seed taken from lemon fruit infected with brown rot*

Water temperature (°F)	Immersion time (minutes)	% recovery *P. citrophthora*	% recovery *P. parasitica*	
75	4	90	100	
105	30	30	100	
108	5	20	90	
108	10	0	60	110°F
108	20	0	70	
120	4	0	0	
125	4	0	0	
130	4	0	0	

Tree sites for the projected orchard that are not on virgin soil or soil not previously cropped to citrus should be treated with Vapam (39.6% sodium *N*-monomethyldithiocarbamate dihydrate) or Mylone (3,5-dimethyltetrahydro-1,3,5-2*H*-thiadiazine-2-thione) to assure at least temporary absence of these fungus root parasites and to give the tree an unhampered start (11). An 8-ft. circular basin site requires a pint of Vapam. The chemical is placed in a pail or other container at the center of the site, and water is run

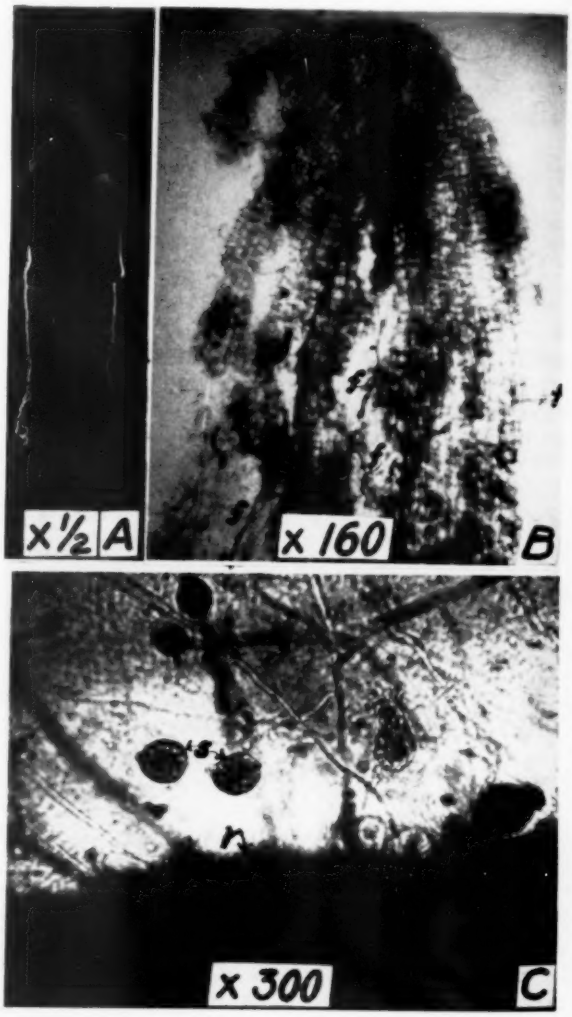

FIG. 4. A) Healthy seedling (left) and seedling with rootlets infected by *Phytophthora parasitica*; B) rootlet from A (right) showing hyphae (f) of *Phytophthora parasitica* in tissue and on surface; C) citrus rootlet (r) with hyphae developing zoosporangia (s) of *Phytophthora* sp.

TABLE 6.—*Increase of trunk diameter (mm), Nov. 19, 1956, to Dec. 19, 1957, from pretreatment of planting sites for protection of citrus nursery trees from root rot by Phytophthora spp. (Prior Lisbon lemon on Sampson Tangelo rootstock)*

Vapam,[a] shavings,[b] no Trichoderma	Vapam, shavings, Trichoderma[c]	Vapam, Trichoderma, no shavings	Vapam, no shavings, no Trichoderma	No Vapam, no shavings, no Trichoderma
16.1[d]	17.5	16.1	16.8	5.4
17.9	13.0	13.6	19.1	6.0
19.1	18.0	10.2	16.3	3.3
15.4	15.8	18.6	10.3	12.3
19.4	12.8	20.4	20.1	5.1
17.0	19.9	6.4	10.5	7.8
18.6	9.1	17.7	14.6	13.5
21.5	20.5	7.4	11.9	5.6
6.2	19.3	19.3	18.2	3.5
11.4	20.8	20.2	11.7	2.0
				5.3
				1.9
				3.5
				6.9
				11.7
Average 16.3*	16.7*	15.0*	15.0*	6.3*

*Increased growth resulting from Vapam pretreatment significant at .001 level. Shavings and *Trichoderma lignorum* had no significant effect on growth.

[a] One pint of Vapam in water of each 8-ft. circular planting site.

[b] 0.5 cu. ft. loose coniferous shavings mixed with soil of planting hole.

[c] Spore-mycelium suspension sprayed on ball of tree and soil-shavings[b] mixture.

[d] Measurements made on trunk at mark 2 in. above standard paper wrapper.

into the container and allowed to overflow into the basin until the Vapam is carried to a depth of 3–4 ft. This can usually be accomplished by using 4–6 in. of water in the basin, depending on the texture of the soil. In another method, the chemical, diluted with water sufficient to carry it to a 3–4-ft. depth, is sprinkled on the soil. Where the whole area is to be treated, the Vapam may be metered into a sprinkling system. The objective is to destroy the fungi from the surface downward. Mylone has an action in soil similar to that of Vapam, and is preferred by some because of its powder form. As a mixture of 50% active ingredient and bran it is distributed at the rate of 800 lb./A. (400 lb. of active ingredient) on the soil surface and raked or rototilled into the upper 2–3 in. of soil. The quantity needed for an 8-ft. circular planting site is 15 oz. Water is then added to take the disinfestant to a depth of 3–4 ft. Sites can be planted with safety 1 month after treatment with Vapam or Mylone.

The fungus *Trichoderma lignorum* (Tode) Harz was shown by Weindling (17) not only to be parasitic on *Phytophthora* spp. but to produce an antibiotic that inhibits the growth of those root parasites. In the following experiments[5] (Tables 6 and 7) a suspension of mycelium and spores was sprayed on the ball of the nursery tree and on the soil and coniferous wood shavings used in planting the tree. The shavings were used on the assumption that they would improve the structure and aeration of the soil and promote the growth of organisms antagonistic to *Phytophthora* spp., thus favoring root growth and health. The results show that *T. lignorum* and/or shavings had little effect on the growth in diameter of Lisbon lemon trunks. Treatment of the planting sites with Vapam, however, was responsible for a 138% increase in growth.

In another pretreatment experiment, in San Diego County,[6] with Lisbon lemon on sour orange rootstock, 30 planting sites were treated with Vapam, and 30 with Mylone, and 5 were left untreated. Reinfestation of the sites by contaminated run-off water caused a variation in the results, precluding statistical analysis, but the benefits 12 months after treatment were indicated by the average circumference of the lemon trunk at a mark 6 in. above the highest point of the bud union: untreated, 51.9 mm; Vapam applied in February, 60.3 mm; Vapam in March, 65.5 mm; Vapam in February and March, 66.6 mm; Mylone in February, 64.2 mm; Mylone in March, 60.5; Mylone in February and March, 67.2 mm.

Contaminated run-off water from rains or irrigation may in some locations enter disinfested basins and recontaminate them. To help prevent this, the soil surface in the basins may be dusted with single-package spray-dried (reacted) Bordeaux mixture or other neutral copper-spray material as soon as the water added with the disinfestants has penetrated the soil. It would be well to keep the surface slightly blue by repeated dustings during the first 2 years of growth. While the fungi of Phytophthora gummosis and root rot will eventually invade the treated tree

[5] Many thanks are due to P. W. Tully, owner of the orchards, and to M. P. Miller and his assistants of the Riverside County Farm Advisors' Office for assistance with these field trials.

[6] The authors acknowledge with thanks the permission of Mr. Tushak, owner of the orchard, and the assistance of C. D. Gustafson and D. O. Rosedale, Farm Advisors of San Diego County, in setting up this experiment.

TABLE 7.—*Effect of wood shavings*[a] *and Trichoderma lignorum*[b] *on increase of trunk diameter (mm) of Prior Lisbon lemon on Sampson Tangelo rootstock (Oct. 1956, to Nov., 1957)*

Replicate	No shavings, no Trichoderma	Shavings, no Trichoderma	Trichoderma, no shavings	Trichoderma, shavings
1	19.21[c]	18.76	19.23	19.55
2	21.82	20.94	20.25	20.50
3	19.05	19.63	21.10	20.79
4	20.72	20.01	21.57	20.00
5	21.27	19.67	19.90	17.96
6	19.75	19.80	20.83	22.45
Treatment Mean	20.30[d]	19.80	20.48	20.21

[a] 0.5 cu. ft. loose coniferous shavings mixed with soil of planting hole.

[b] Spore-mycelium suspension sprayed on ball of tree and soil-shavings[a] mixture.

[c] Average of 23 trees; measurements made on trunk at mark 2 in. above standard paper wrapper.

[d] Average of 138 trees.

sites, the young trees will have time to become established before this occurs. Trees surgically treated for Phytophthora gummosis 11 years ago have since been protected from recurrence of infection by dusting the soil around the crown twice yearly (Klotz, unpublished). Under good cultural conditions, trees can grow fibrous roots faster than the fungi can destroy them. Good cultural methods pertinent to root health include tillage or non-tillage methods and weed control that do not injure roots, not overwatering, and not overfertilizing with organic and inorganic materials.

Where it is necessary to water young trees from a tank wagon during the first 2 years of growth, any water contaminated with *Phytophthora* spp. should be disinfested with 20 ppm copper sulfate (about 1 oz. $CuSO_4 \cdot 5H_2O$ in 400 gal.) or 10 ppm of malachite green (1 oz. in 400 gal.). Sources of canal and reservoir water are being tested for infestation with these fungi. Of 14 sources of water tested thus far, 11 have been found infested by *Phytophthora* spp. Such contaminated water should be treated with copper sulfate. The quantity of run-off water should be minimized by good regulation of irrigation, and any excess water should be run through leak-proof conduits into reservoirs or sumps, where it can be disinfested with copper sulfate before re-use.

The ideal way to avoid the deterioration of citrus trees from fibrous root rot is to use a rootstock resistant to the *Phytophthora* spp. under all or most cultural conditions. As already shown, citrus species and varieties vary greatly in their resistance to this type of root rot, but none so far tested is completely immune. The most resistant species tested was the trifoliate orange, *Poncirus trifoliata*. Some selections of trifoliate orange showed marked resistance even in solution culture. They have the added advantages of being tolerant to the destructive virus disease, Tristeza, and of doing well even in heavy soils that dry out with difficulty. Disadvantages of the trifoliate orange are the slow germination of its seeds and the susceptibility of the rootstocks to the exocortis virus, present in much commercial scion material. Only budwood sources known to be free of this disease factor should be used. Nucellar trees are free of it until infected from an outside source. Because of the present dearth of such budwood sources it is necessary to follow the procedures outlined above to establish a successful citrus orchard on the kinds of rootstocks generally used.

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URANIUM AND SPORE RESPIRATION IN NEUROSPORA SITOPHILA[1]

Vincent W. Cochrane and Dade L. W. Tull[2]

SUMMARY

Uranyl nitrate abolishes the uptake of oxygen associated with glucose utilization by conidia of *Neurospora sitophila;* the approximate ED_{50} is 1.0 μg per 10^5 spores. Endogenous oxygen uptake is not, however, affected by concentrations of uranyl nitrate 30 times as great. In the presence of uranium the respiratory quotient with glucose drops to the endogenous level. Phosphate reverses the uranium effect on glucose respiration. Anaerobic glucose respiration is about 20 times as sensitive to uranium as is aerobic respiration. The oxidation of acetate and ethanol is little affected by uranium at a concentration inhibiting over 80 per cent of glucose respiration. These effects of uranyl ion on glucose respiration are ascribed to a blocking of the uptake of glucose at or near the surface of the spore. Mercuric chloride at very low concentration inhibits the oxidation of glucose more than it inhibits endogenous respiration, but, unlike uranyl ion, even slightly higher concentrations abolish all respiratory activity. The data in general emphasize that any assay of fungitoxicity based on respiratory responses must take into account the substrate of respiration.

Rothstein (14) reviewed an extensive series of studies on the action of uranyl ion on yeast, and concluded that its primary effect is inhibition of hexose metabolism, exerted at or near the cell surface. Uranyl ion at concentrations that inhibit glucose metabolism of yeast has little or no effect on endogenous respiration or on the metabolism of substrates other than hexoses.

Mercury and other cationic heavy metals are usually regarded as general enzyme poisons, and these metals appear to enter fungus spores extremely rapidly (9, 11). However, these is some evidence for specific effects of mercury on the transport of phosphate in bacteria (12) and on the uptake of glucose by rat diaphragm (2).

The purpose of the present work is, first, to determine if the action of uranyl ion is the same on fungus spores as on yeast (14). If so, the method of demonstration might prove applicable to the cationic heavy metals, e.g., mercury. It is conceivable that the first action of these heavy metals occurs at or near the cell surface, and that only after the permeability barrier has broken down does the cation enter the cell.

Although a glucose uptake experiment has been included, major reliance has been placed on spore respiration. We assume that, if a toxicant inhibits oxidation of one substrate but not that of others or of endogenous reserves within the cell, it is acting at or near the cell surface and exerts its effects primarily on the uptake of the substrate. Uranium is an example (14). Conversely, if a toxicant inhibits all respiration, including endogenous oxidations, it seems a fair inference that this material is acting as a general inhibitor of metabolism, or at least exerts its effect on some late stage in the respiratory process through which all respiration must pass. The antibiotic nystatin, acting on yeast and on *Pencillium chrysogenum*, is a good example of this second type of inhibitor (5).

METHODS.—An isolate of *Neurospora sitophila* Shear & Dodge, kindly given us by Dr. S. E. A. McCallan, was grown for spore production on the complex agar medium of Horowitz (3). After 2–4 days at room temperature, the cotton plugs were removed and replaced by a single layer of cheesecloth; at the same time the flasks were put under constant low-intensity artificial light. Spore formation usually proceeds rapidly under these conditions, and spores were harvested 36–48 hours after removal of the plugs.

Conidia were washed from the agar, passed through a double layer of cheesecloth, centrifuged down, resuspended in distilled water, centrifuged again, resuspended, and filtered off on a "Millipore" filter, Type RA (pore size 1.2 μ). The number of spores was determined in a Klett colorimeter using a green (540) filter; the instrument was calibrated against hemacytometer counts of spores.

Since uranyl ion complexes with phosphate, carboxyl, and hydroxyl ions, and since the extent of this complexing is negligible only at rather low pH (15), all experiments, except as noted, were carried out at pH 3.5 and in the absence of exogenous phosphate. Gas exchange was determined at 30°C by conventional manometric methods (18) except as noted. C^{14}-labeled carbon dioxide collected in the center well of a Warburg flask was passed into standardized $Ba(OH)_2$ and counted as $BaCO_3$. Glucose was determined by the anthrone method (17).

RESULTS.—Since a separation between endogenous and substrate respiration is essential to the basic experimental design, it was necessary to see first if the rate of endogenous respiration is affected by added exogenous substrate. The method used is essentially that of Barker (1). Oxygen uptake at graded substrate levels is determined; if the response to glucose is linear after substraction of the endogenous oxygen uptake (determined as usual in duplicate control flasks lacking substrate), it is concluded that external substrate does not influence the oxidation of reserve

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[2] Professor of Biology and Graduate Assistant, respectively.

TABLE 1.—*The effect of phosphate and acidity on spore respiration*

pH	Phosphate	Oxygen uptake [a]	
		Endogenous	Exogenous [b]
5.5	0.0	1.1	1.8
3.5	0.0	0.94	3.2
5.5	0.01 *M*	0.92	1.6
3.5	0.01 *M*	1.1	2.7

[a] Reported as μl per 10^5 spores per hr.
[b] Oxygen uptake with glucose (20 μM per flask), less endogenous.

substances. The data plotted in Fig. 1 show not only that corrected oxygen uptake is a linear function of glucose concentration, but also that extrapolation to zero substrate yields a value that agrees fairly well with the experimentally determined endogenous respiration. We conclude that there may be a slight suppression of endogenous respiration by added substrate, but that it is not accurately measurable with this technique and is not large enough to affect the validity of other results to be reported.

It was also necessary to determine if the unusual conditions—low pH and absence of phosphate—seriously affect spore respiration. The data of Table 1 indicate that endogenous respiration is little affected; surprisingly enough, glucose respiration is actually more rapid at pH 3.5 than at 5.5.

In Fig. 2 the major characteristics of uranium inhibition are evident. Endogenous respiration is not appreciably affected, but the extra oxygen consumption normally associated with glucose utilization is suppressed.

Fig. 3 is made up from data of 9 separate experiments, and allows the calculation that the ED_{50} for uranium inhibition of glucose respiration is 1.0 μg uranyl nitrate (0.47 μg uranium) per 10^5 spores. Endogenous respiration is not inhibited by a concentration of 30 times the ED_{50} value. Indeed, at these high concentrations of uranium there is often a marked stimulation of endogenous respiration, but the effect is not fully reproducible and has not been further studied. Stimulation of respiration by toxic agents has of course been reported by others (7).

The effect of uranium on carbon dioxide evolution was determined in 2 ways, directly by measuring $C^{14}O_2$ liberation from glucose uniformly labeled with carbon-14, and indirectly by determination of respiratory quotients.

The data of Table 2 show that aerobic carbon dioxide evolution is suppressed by uranium. The manometric and radiochemical yields do not agree in detail, nor is the suppression of carbon dioxide formation apparently so complete as the inhibition of oxygen uptake in previous experiments.

Table 3 demonstrates that the respiratory quotient in the uranium-glucose system is the same as that in the uranium–no-substrate system. That is, the effect of uranium is to abolish the response to glucose. The respiratory quotient for normal unpoisoned spores in

TABLE 2.—*The effect of uranium on carbon dioxide liberation*

Exp. no.	System	Per cent yield	
		Manometric [a]	Radiochemical [b]
1	Glucose-U-C^{14}	9.2	8.9
	Same, plus uranyl nitrate 1.4 μg. per 10^5 spores	2.3	0.4
2	Glucose-U-C^{14}	13.3	8.4
	Same, plus uranyl nitrate 4.1 μg. per 10^5 spores	4.3	1.8

[a] Carbon dioxide as per cent of the theoretical maximum obtainable from the glucose carbon supplied; determined by injection of alkali into a sidearm after completion of the experimental period.
[b] $C^{14}O_2$ as per cent of the theoretical maximum obtainable from the uniformly labeled glucose (glucose-U-C^{14}) supplied.

the absence of external substrate agrees closely with the value earlier determined by Owens (13).

One minor point, the slight increase in respiratory quotient apparently caused by uranium, must remain unclear. This effect is at the borderline of significance, considering the accuracy of the manometric apparatus used, representing only a few mm per hour observed pressure change. Should more precise measurement prove this effect real, some influence of uranium on endogenous carbon dioxide liberation will have to be explained.

Reversal of the uranium effect by phosphate is shown in Fig. 4. The proposed explanation for this phenomenon is that phosphate and uranium compete for sites on the cell surface (15). The rapidity of the reversal, evident in the original data after only 10 minutes, argues for a rapid replacement of uranium by phosphate and, consequently, for a surface locus of the competition, since such rapid penetration of an anion into the interior of the cell seems unlikely.

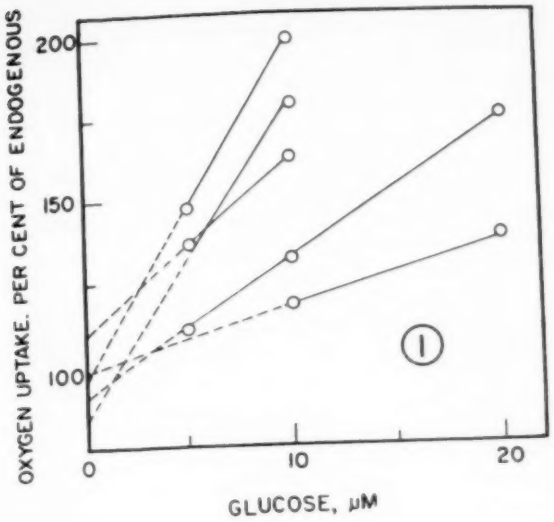
Again as in yeast (16), anaerobic fermentation by conidia of *N. sitophila* is considerably more sensitive to uranium than is aerobic oxygen uptake (Table 4). A rough comparison with Fig. 2 suggests that the ED_{50} for inhibition of fermentation is about $\frac{1}{20}$ of the ED_{50} for inhibition of aerobic respiration.

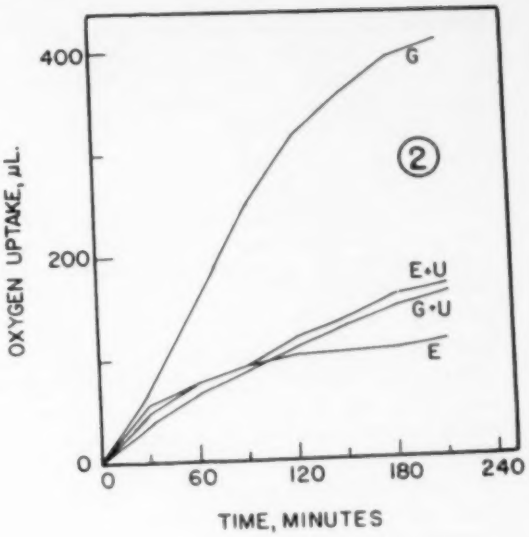
Table 5 summarizes data on the oxidation of acetate

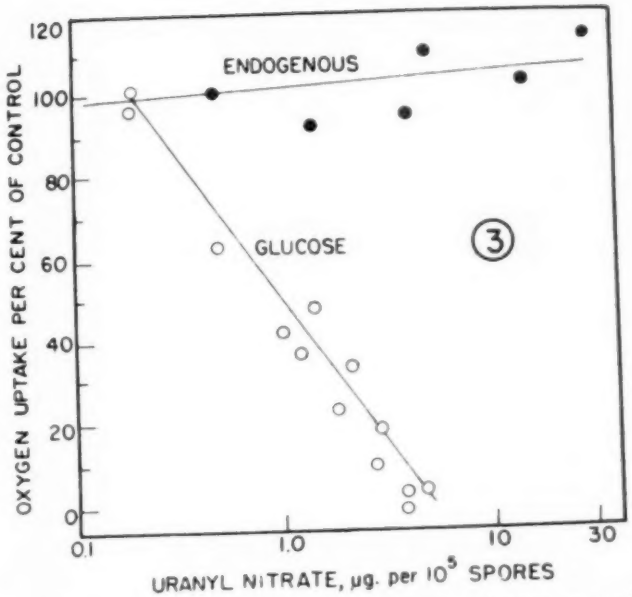
TABLE 3.—*Uranium and the respiratory quotient*

System	Oxygen uptake [b]	CO_2 output [b]	Respiratory quotient
No substrate	1.74	1.24	0.71
No substrate, uranyl nitrate [c]	1.60	1.38	0.86
Glucose, 20 μM	4.35	4.59	1.06
Glucose, 20 μM, uranyl nitrate [c]	2.44	2.11	0.86

[a] μg per 10^5 spores.
[b] μl per 10^5 spores per 2 hr.
[c] 1.4 μg per 10^5 spores.







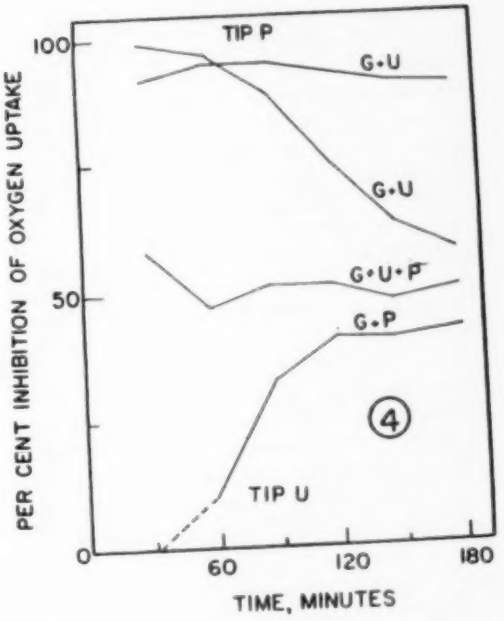


FIG. 1–4. Fig. 1. The effect of glucose as substrate on respiration. Data plotted in this form give, when extrapolated to zero glucose (dashed line), a calculated endogenous value; for each experiment the observed endogenous oxygen uptake was set as 100%, and oxygen uptake with substrate was computed to this base. Each curve represents a different population of spores.—Fig. 2. The effect of uranyl nitrate on endogenous and glucose respirative. E = endogenous, G = glucose (20 μM), U = uranyl nitrate (3.7 μg per 10^5 spores). Each flask contained 80×10^5 spores, $MgCl_2$ (0.003M), glucose and/or uranyl nitrate as shown, and alkali (center well), in a total fluid volume of 3.2 ml. —Fig. 3. The relative sensitivity of endogenous and substrate (glucose, 20 μM per flask) to uranyl nitrate. The data are from 9 separate experiments.—Fig. 4. Reversal of uranium inhibition by phosphate. G = glucose (20 μM per flask), P = phosphate (1.6 μg K_2PO_4 per 10^5 spores), U = uranyl nitrate (3.3 μg per 10^5 spores). Phosphate and uranium added at 60 minutes to the flasks represented by, respectively, the uppermost and lowermost curves. Each flask contained 82×10^5 spores, 10 μM $MgCl_2$, and the additions shown. Oxygen uptake values are corrected for endogenous respiration, and data are plotted for 6 successive 30-minute periods (0–30, 30–60, etc.).

TABLE 4.—*Uranium and fermentation*

Uranyl nitrate (μg per 10^5 spores)	Inhibition, per cent	
	Aerobic [a]	Anaerobic [b]
4.6	44.6	100
0.46	21.9	71.8
0.046	2.2	56.3

[a] Oxygen uptake, corrected for endogenous; gas phase air, 64×10^5 spores per flask; time, 3 hrs.
[b] Carbon dioxide, no endogenous correction required; gas phase nitrogen, 64×10^5 spores per flask; time, 3 hrs.

and ethanol in the presence and absence of uranium sufficient to inhibit glucose oxidation over 80 per cent. It is seen that uranyl nitrate exerts only a small effect on the oxidation of these 2-carbon compounds. This is further evidence that the effect of uranyl ion at this concentration is specifically on glucose metabolism and not on any metabolic system of general importance to respiration.

Finally, Table 6 shows more directly that glucose uptake is inhibited by uranium. In this experiment, a large volume of spores was pretreated for 15 minutes with uranyl acetate and then incubated 15 minutes with glucose; controls were pretreated with sodium acetate. The spores were then centrifuged down, and residual glucose was determined in the supernatant. About 70 per cent of the normal glucose uptake was abolished by the poison at a concentration known from earlier experiments (Fig. 1, 2) to inhibit glucose respiration without affecting endogenous oxygen uptake. We conclude that the inhibition of respiration is in fact an inhibition of uptake.

Preliminary experiments on mercuric chloride inhibition of respiration reveal a situation that may be similar to uranium inhibition but is experimentally more difficult to study. At a low toxicant concentration, 4.4 μg $HgCl_2$ per 10^5 spores, substrate respiration is more inhibited than is endogenous (Fig. 5). With endogenous values subtracted, Fig. 5 shows that glucose respiration is inhibited about 78 per cent. However, there is also a significant—about 14 per cent—inhibition of endogenous respiration.

In later experiments it was found that, although the effect of mercury on endogenous respiration is somewhat less than its effect on glucose respiration, the difference is small, not at all comparable to that found for uranium. Consequently, it is not possible to apply respiratory data of this scale of precision to the question of the first locus of mercury toxicity. Accurate respiratory measurements in the first few minutes of contact between cell and poison conceivably would give unambiguous answers.

Thus, although the data of Fig. 5 suggest that there is a concentration of mercury that acts primarily on glucose uptake rather than on the internal enzymatic reactions of endogenous respiration, no firm conclusion is possible at this time.

DISCUSSION.—In agreement with Rothstein (14), who bases his conclusions on work with yeast, we propose that the action of uranyl nitrate on spore respiration in *Neurospora sitophila* is by interference with the uptake of glucose, and that this effect is exerted at or near the cell surface. The principal bases for this hypothesis are:

1) Glucose oxidation is abolished by concentrations of uranium that have no effect on endogenous respiration and no significant effect on the oxidation of acetate or ethanol.

2) The respiratory quotient is lowered by uranium to the value characteristic of endogenous respiration, i.e., in the presence of the uranium, glucose does not exert its usual metabolic effect.

3) Uranium inhibition is rapidly reversed by phosphate.

4) The uptake of glucose by spores is inhibited under experimental conditions that afford a direct measurement of the removal of glucose from solution by spores.

The argument for a similar action of very low concentrations of mercury is possible, but, as briefly discussed above, the proof is more difficult; a relatively small increase in the concentration of mercury changes the picture to one of complete inhibition of all respiratory activity. Copper, silver, and mercury offer still another experimental difficulty, in that low concentrations considerably stimulate endogenous respiration (unpublished experiments).

It has not been possible to germinate washed spores of *N. sitophila* under the conditions used in our respirometric experiments; consequently, direct comparison with fungistatic data is not possible. McCallan and Wellman (8), using rather different conditions, report the fungistatic ED_{50} for uranium as about

TABLE 5.—*Uranium and substrate oxidations*

System	Oxygen uptake [b]	Per cent inhibition
Glucose, 0.1 *M*, no uranium	1.54	
Glucose, 0.1 *M*, plus uranyl nitrate [a]	0.24	84
Acetate, 0.1 *M*, no uranium	1.71	
Acetate, 0.1 *M*, plus uranyl nitrate [a]	1.50	12
Ethanol, 0.1 *M*, no uranium	1.62	
Ethanol, 0.1*M*, plus uranyl nitrate [a]	1.78	−10

[a] μg per 10^5 spores.
[b] μl per 10^5 spores per 2 hr., corrected for endogenous respiration.

TABLE 6.—*Uranium and glucose uptake*

	Control [a]	Uranyl acetate [b]
Glucose supplied, μg	1440	1440
Glucose recovered, μg	693	1227
Glucose removed, μg	747	213
Per cent removal	51.9	14.8
Inhibition, per cent		71.5

[a] Control system: 9.1×10^8 spores (dry weight 327.8 mg), sodium acetate, 271.6 μM, glucose as shown, pH 3.5, total volume 48 ml. Time of exposure to glucose 15 min.
[b] Like control, but uranyl acetate 135.8 μM (3.55 μg uranium per 10^5 spores) in place of sodium acetate.

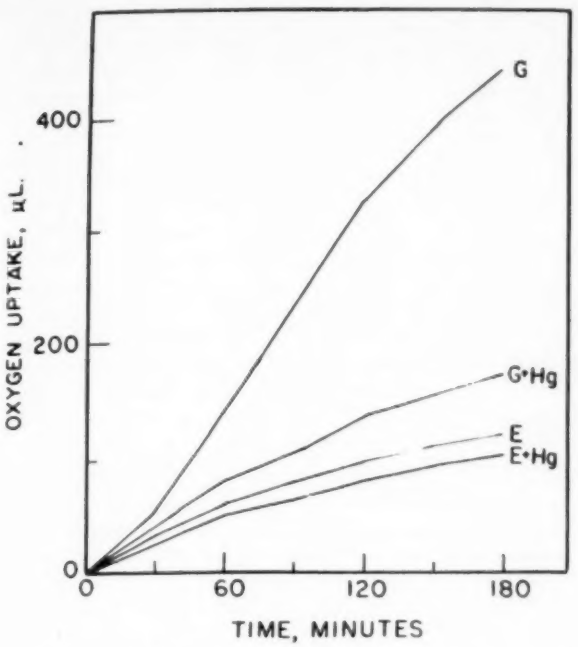


FIG. 5. The effect of mercuric chloride (4.4 μg per 10^5 spores) on respiration of conidia of *Neurospora sitophila*. G = glucose (20 μM per flask), E = endogenous, Hg = mercuric chloride.

0.42 ppm for *Monilinia fructicola*, the most sensitive of 4 fungi tested. This is a bulk concentration, and is an order of magnitude lower than the ED_{50} found in Fig. 3 (computed on the basis of external concentration).

These results have some application to the question of whether or not the inhibition of respiration can serve as an index of fungitoxicity. We must first note that the 2 elements studied, uranium and mercury, are rather unlike in their fungistatic and fungicidal potency (8). Their effects in glucose oxidation are not nearly so dissimilar. As noted earlier, in discussion of Fig. 3, the ED_{50} for inhibition of glucose respiration is 0.47 μg uranium per 10^5 spores. We have found in unpublished experiments that the ED_{50} for mercury inhibition of glucose oxidation is actually somewhat higher—about 1.1 μg mercury per 10^5 spores. This is of course in contrast to the relative position of the elements on the scale of fungistatic activity (8). The unusual conditions—low pH and absence of phosphate—preclude direct comparison with the literature. Thus, our ED_{50} of 1.1 μg is about 4 times as great as the equivalent value for inhibition of total respiration in the presence of sucrose, which can be calculated from the data of McCallan, Miller, and Weed (7) on the basis that *N. sitophila* spores have a fresh weight of 0.35 mg per 10^6 spores (10). The difference may well reflect competition of hydrogen ion with mercury in our experiments.

Second, it is clear that respiratory responses are specific in the sense that the substrate being oxidized influences the response. Uranium is more effective than mercury against glucose respiration, and much less effective against endogenous respiration. Given this specificity, it is difficult at present to compare data from different laboratories; thus, Klöpping (4) measured the effect of fungistatic agents on endogenous respiration, whereas McCallan, Miller, and Weed (7) included sucrose in the respiration medium.

We may speculate that respiration is perhaps not the method of choice for an assay of fungitoxicity. If it is so to be used, 2 conditions must be met: the response must be on the basis of toxicant actually taken up (6, 11), and the response should be measured both in the presence and the absence of exogenous substrate.

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THE NATURE AND INHERITANCE OF RESISTANCE TO THE PEA ENATION MOSAIC VIRUS IN GARDEN PEA, PISUM SATIVUM L.[1]

W. T. Schroeder and D. W. Barton

SUMMARY

Resistance to PEMV (pea enation mosaic virus) was obtained by selection from P. I. 140295. The selection, G168, used as a parent in crosses with susceptible domestic varieties, is not immune from the virus; atypical symptoms developed as a faint mottle and chlorotic flecking. Some plants showed no symptoms. Virus recovery from inoculated resistant plants was independent of the severity of the atypical syndrome. Resistant plants reacted similarly to cultures of PEMV from different pea-growing areas in the United States. On rare occasions, observed only with the greenhouse inoculation technique, a resistant plant developed typical enation symptoms at bloom or later. Isolates from such plants were capable of producing typical symptoms in resistant plants, but their virulence seemed to diminish after maintenance on susceptible pea plants. Genetically, resistance, as described, was conditioned by a single dominant gene not closely linked with Le or R.

INTRODUCTION.—Pea enation mosaic (PEMV) is the most important virus disease of processing peas (*Pisum sativum* L.) in New York State, in many instances accounting for 75% or more of the total viroses. Although economic gains in yield through application of insecticides to control virus vectors have been demonstrated in many instances (7), it is doubtful whether such a practice would be economically practical on the over-all acreage year after year. It would appear, therefore, that some sort of heritable resistance to this disease would be the best solution of the problem.

Before 1950, no clear-cut resistance to the damaging effect of enation mosaic had been demonstrated, although some varieties were reported to be less adversely affected than others (6, 8). In 1950 Schroeder (5) grew 171 foreign introductions and 47 domestic pea varieties in a field trellis planting, and found all but one completely susceptible to pea enation mosaic. In that one (P.I. 140295), 4 plants out of 301 remained free of the disease, and subsequent progenies were designated G168. Seeds from each of those 4 plants were saved separately for testing in the greenhouse with a culture of the virus obtained from an infected plant in that field. Sufficient resistance had been found to warrant a breeding program to incorporate such resistance into commercially acceptable varieties. Studies on the nature of this resistance and its inheritance are herein reported.

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MATERIALS AND METHODS.—The following isolates of PEMV, obtained from different pea-growing areas of the country, were used in these studies: PA-6 (New York), PB-2 (Washington) and PM-1 (Oregon).

Test plants were inoculated by means of aphids (*Macrosiphum pisi* Kalt.) reared on infected pea plants. Three viruliferous aphids, placed on each test plant at the 1–2 true-leaf stage, were allowed to feed for 24 hours and then killed by fumigation. After 2 days, 3 other viruliferous aphids were placed on each plant, allowed to feed 6–24 hours, and killed. This method was superior to mechanical inoculation, resulting in typical pea enation mosaic on every plant of the susceptible control variety, Bonneville, in all tests (Fig. 1). Typical pea enation mosaic, the criterion of susceptibility, develops as a mottling and a chlorotic (sometimes necrotic) flecking of newly unfolded leaves, usually accompanied by a twisting and distortion of the apical growth. Within 10–14 days after the last vector transfer, leaf enations and veinal overgrowths develop on the dorsal leaf surface. Later, overgrowths and deformations develop on the pods also. The presence of enations, overgrowths, and apical distortions on the young plants was essential before a plant was judged susceptible since they are the symptoms that later result in damage to the plant.

Parental materials were the uniformly resistant selection from P.I. 140295, maintained as G168, and various susceptible domestic varieties, such as Perfection, Surprise, Pluperfect, and Perfected Freezer.

Plants of G168 attain a vine length of 60 in. in the field under good growing conditions. The flowers are

white, and double flowers and double pods per node are frequent above the 14th to 16th nodes. The peas are of medium size, the seed is round indent with green cotyledons. The eating quality of the green peas is quite poor. In contrast, most of the PEMV-susceptible domestic pea varieties are dwarf, with a maximum vine length of 36–40 in. in the field. The varieties used for canning and freezing in New York have wrinkled seeds.

All crossing and progeny testing were done in the greenhouse.

NATURE OF RESISTANCE.—*Symptomatology and virus recovery in resistant plants.*—During the initial testing of G168, some plants developed symptoms of PEMV (though atypical), indicating that resistance in this line might not be complete or absolute. Many of the plants from the G168 selections inoculated with the PA-6 culture of the virus showed a light to dark green mottle on the newly expanded leaflet, often accompanied by the suggestion of a dark green banding of the midrib. Following this atypical symptom, slight chlorotic flecks appeared on the same leaves and succeeding ones, indicating a systemic movement of the virus (Fig. 2). On some plants, however, the chlorotic flecks appeared on the leaves of only a few nodes and then disappeared. The rate of growth of the inoculated resistant plants was not impeded, and they were, aside from the slight chlorotic flecking, as normal as the uninoculated controls. Still other plants showed no symptoms at all. The development of atypical symptoms has not been observed on resistant plants under natural field infection.

The production of atypical symptoms on G168, as well as on resistant breeding progeny, was not confined to the virus culture PA-6. Other PEMV isolates from peas in western New York and virus cultures from Washington and Oregon also incited a mild foliar reaction. Some of these isolates caused a slightly more pronounced atypical syndrome in some greenhouse tests, but it was impossible to demonstrate that any one of the PEMV isolates studied was consistently any more or less virulent than another.

The severity of atypical symptoms, however, did not affect the ease of virus recovery from such plants via vector transfers to the susceptible Bonneville plants. In one experiment, 43 plants of the resistant line were vector-inoculated with isolate PA-6, and 43 were similarly inoculated with isolate PB-2. Two groups of 12 susceptible New Era plants were each inoculated with isolate PB-2. Two groups of 12 susceptible New Era plants were each inoculated with the above isolates, and all developed typical enation symptoms. All of the inoculated resistant plants, irrespective of the virus isolate, developed systemic atypical symptoms. The symptoms on plants inoculated with PA-6 were very pronounced, whereas those on plants inoculated with PB-2 were barely perceptible. Virus recovery attempts were made on 20 plants in each group by allowing healthy aphids to feed for a prescribed period on each plant and then transferring 3 aphids

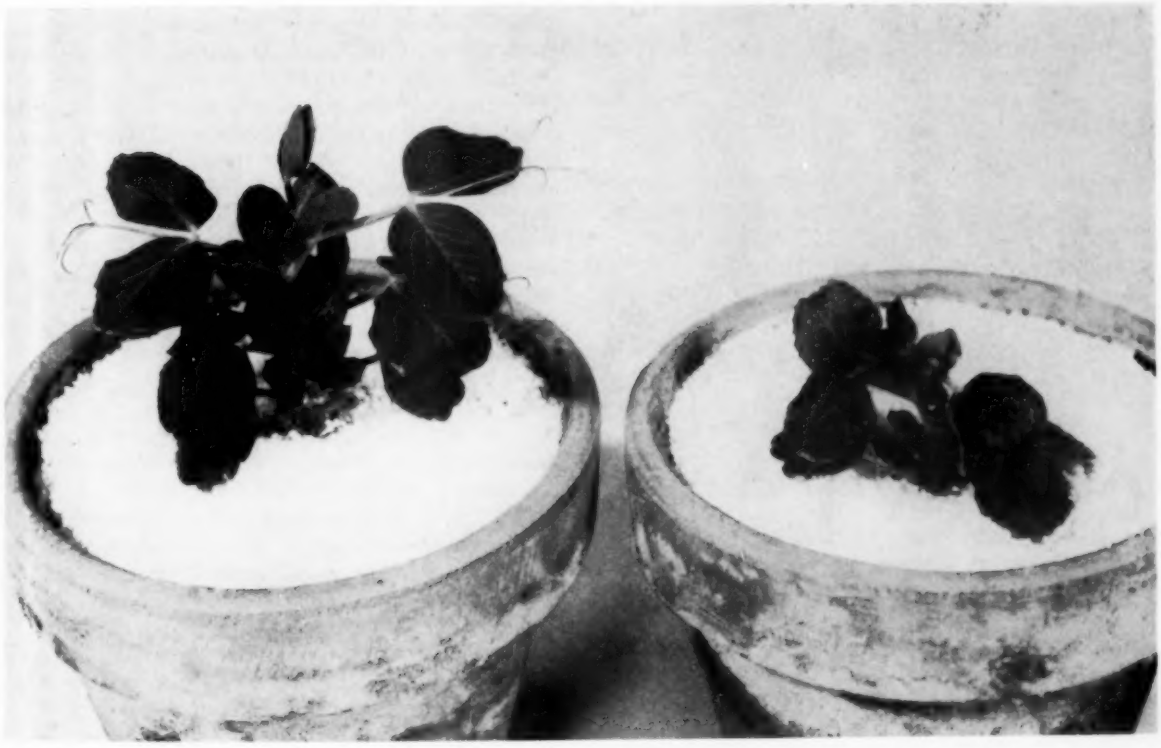

FIG. 1. Resistant plant (left) and susceptible plant (right) about 2 weeks after last vector transfer.

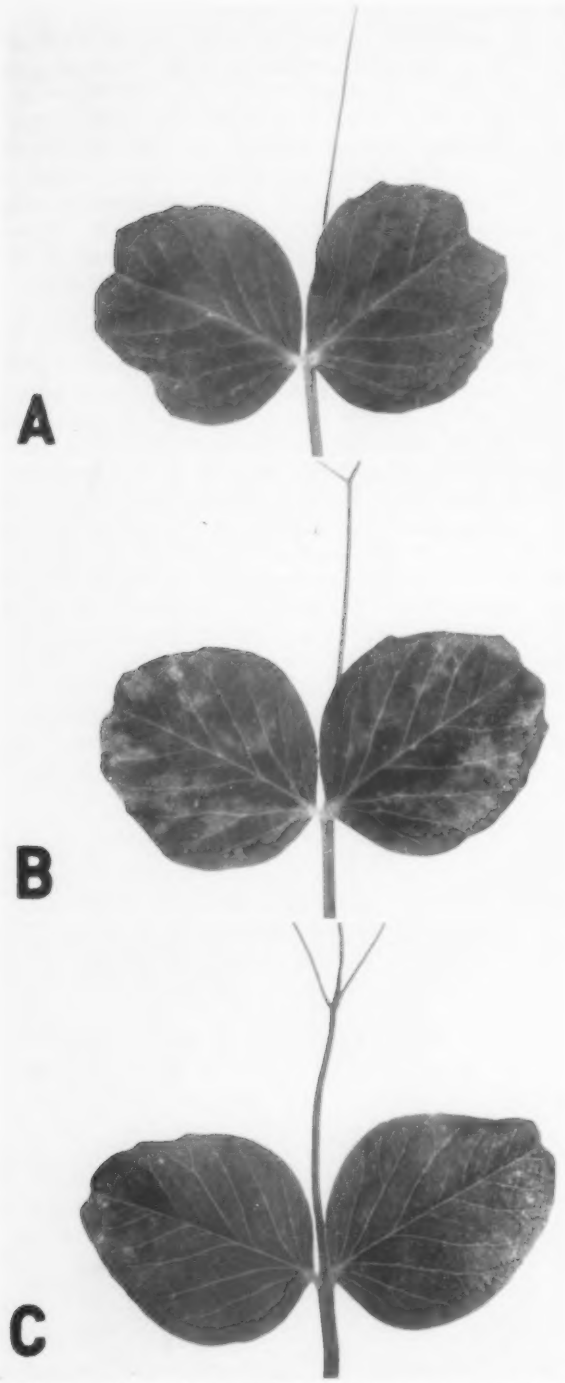

FIG. 2. Atypical symptom expression on 3 successive leaves of a G168 plant following aphid transfer of PEMV. A) Leaf that unfolded during or shortly after the feeding period, showing no abnormality. B) Next younger leaf shows a light-dark-green mottle and a suggestion of a broad vein-banding of midrib, accompanied by a few chlorotic flecks. C) Succeeding leaf shows only the slight chlorotic flecking characteristic of the reaction of resistant plants to the virus.

to each of 5 susceptible Bonneville plants. No virus was recovered from the plants inoculated with PA-6, although they had the most pronounced atypical symptoms; whereas, from 6 of the 20 plants inoculated with PB-2, virus was recovered that produced typical symptoms on the susceptible Bonneville. The virus was recovered from 100% of the inoculated New Era controls.

The behavior of the isolates in greenhouse inoculations was not consistent. In a similar experiment involving isolates PA-6 and PM-1, some of the inoculated resistant plants were normal in appearance whereas others had the systemic atypical symptoms, regardless of the inoculant. No virus was recovered from plants inoculated with PM-1; from plants inoculated with PA-6, the virus was recovered from 2 of 18 showing atypical symptoms and from 1 of 4 plants appearing symptomless. These results indicate little direct relationship between the severity of atypical symptoms and virus recovery by aphids.

The development of new virus strains after a breeding program is well under way is always possible, and occurred in this instance. In testing thousands of breeding progeny in the greenhouse, using the vector transfer method, almost all of the susceptible plants were eliminated within 2 weeks after inoculation. The resistant selections flowered and set pods in a normal fashion, except for a few plants. In these exceptions, typical enation symptoms suddenly appeared at or after the blossoming period. Virus cultures from these plants were isolated, and vector transfers were made to both susceptible and resistant lines. In the susceptible lines the symptoms did not appear to be quite as severe from the aberrant isolates as from PA-6, PB-2 and PM-1. In some of the resistant plants typical enation symptoms appeared after inoculation with these aberrant isolates, and the virus was recovered from all inoculated plants. It is interesting to note, however, that when one of these isolates was maintained on susceptible pea plants for several transfers it lost its virulence for the resistant lines. It should be pointed out here that the appearance of this aberrant strain, if it can be called such, has never been observed in the field, and only in a few instances out of thousands of transfers in the greenhouse.

On the basis of the above experiments and observations, it appears that resistance depends on a rather delicate relationship, as yet undetermined, between the physiological forces of the virus within the plant and those of the host.

The genetic basis of enation virus resistance.—Insofar as crosses between G168 and domestic varieties are concerned, the basic gene differences are tall versus dwarf and round seed versus wrinkled seed. The gene for tallness is designated Le and is in linkage group IV (2), whereas that for round seed is R, in linkage group VII (1). Exceptions to the above occur in crosses between G168 and the early varieties, Surprise and Thomas Laxton, which are, like G168, genetically tall.

TABLE 1.—*F_2 segregations for resistance and susceptibility to enation mosaic virus*

Parentage	Total	Resistant		Susceptible		Chi-square		
		Tall En Le[a]	Dwarf En Le	Tall en Le	Dwarf en le[a]	En:en 3:1	Le:le 3:1	9:3:3:1
GH52-3 × G168	56	39	10	5	2	4.03*	.21	4.23
Commando × G168	44	29	5	6	4	.03	.27	2.06
G51-70(1) × G168	39	20	8	9	2	.19	.00	0.28
G51-70(2) × G168	36	20	5	9	2	.33	.33	0.68
Totals	175	108	28	29	10	0.55	0.84	1.72
				Heterogeneity	(3d.f.)	4.03	.00	5.53

[a] Parental types.

Initial crosses of G168 with the varieties Surprise, Perfection, Shoshone, New Era, Commando, Thomas Laxton, Pluperfect, and Perfected Freezer, as well as with several Geneva breeding lines, produced enation-mosaic-resistant F_1 hybrids in all cases. These hybrids had the faint systemic mottling characteristic of G168 after aphid inoculation.

The progeny tests of selfed F_1 plants resulted in segregations of resistant to susceptible plants in a 3:1 ratio (Table 1), indicating that resistance is conditioned by a single dominant gene, hereinafter designated En. One population deviated from a 3:1 ratio at the 5% level of significance, but later tests indicated that this is normal. The segregations of En and Le did not differ significantly from a 9:3:3:1 ratio, indicating that the linkage between En and Le, if such exists, is not close.

Further evidence of the single-gene nature of resistance from G168 is found in the backcrosses of the F_1's to the enation-susceptible parents (Table 2). These results clearly show that PEMV resistance is conditioned by a single dominant gene. The combined segregations for R and En, Le and En, and R and Le correspond favorably with 1:1:1:1 expectations with independent inheritance (Table 3). It should be mentioned that the F_2 segregations for R and r, as well as for the backcross material, were not completely classified, since the R (indent) type is difficult to distinguish from the r (wrinkled) type when the seed has been produced in the greenhouse. This is especially true in cases where heterozygosis for the R gene is involved. For this reason, part of the seed of the F_2 segregations and part of the seed of the backcross segregations were not classified. Only clearly classifiable R segregations are reported in Table 3. In these experiments, no linkage was apparent between En and Le (in linkage group IV), or with R (in linkage group VII).

DISCUSSION.—Resistance to PEMV as defined in this paper appears horticulturally practical, and is conditioned by a single dominant gene. This resistance is not in the nature of immunity but rather in the ability of the plant to grow and reproduce normally even though it may be systemically infected with PEMV. The occasional occurrence of aberrant strains of the virus under the greenhouse inoculation technique employed poses a problem that cannot be ignored. Whether or not such strains occur in the field environment remains to be seen, but they have not been observed in 4 years' field tests of resistant lines grown under conditions of severe natural infection. Evidence that strains of PEMV capable of overcoming resistance do not yet exist in nature in the pea-growing regions of the United States is indicated by the fact that G168 and homozygous-resistant breeding lines sent to various investigators for trial have maintained their resistance (3).

The existence of strains of PEMV have been discussed and even described, but only on the basis of symptom expression in hosts such as *Vicia faba* and on the ease of transmission by mechanical means (3, 4). The studies reported herein suggest that strains more virulent to G168 and progenies derived therefrom can be created by passage through these hosts, but whether or not they would be maintained as such in natural overwintering suscepts should be determined.

The mode of inheritance of resistance is such that

TABLE 2.—*Backcross segregations for resistance and susceptibility to enation mosaic virus—type cross (G168 × susceptible) × susceptible*

Susceptible Parent	Number of Plants			Chi-square 1:1
	Total	Resistant	Susceptible	
Perfection	25	14	11	0.16
New Era	22	11	11	0.00
Shoshone	15	4	11	2.40
Perfected Freezer	39	20	19	0.00
Pluperfect	32	14	18	0.28
Thomas Laxton	53	32	21	1.88
Surprise	57	29	28	0.00
Stratagem	33	13	20	0.96
Totals	276	137	139	0.01
		Heterogeneity	(7d.f.)	5.67

TABLE 3.—*Combined segregations in backcrosses of F_1's to susceptible parents—type cross (AABB × aabb) × aabb*

Tested genes	Total	AB	Ab	aB	ab	Chi-square 1:1:1:1
R—En	145	39	34	29	43	2.56
Le—En	116	25	25	30	36	2.21
R—Le	116	28	31	22	35	2.59

it can be easily employed in a breeding program for incorporation into any desired variety, utilizing the backcross method.

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WHITE TIP DISEASE OF RICE. I. SYMPTOMS, LABORATORY CULTURE OF NEMATODES, AND PATHOGENICITY TESTS[1]

E. H. Todd[2] and John G. Atkins[3]

SUMMARY

White tip has been an important panicle and leaf disease of rice in Louisiana, Arkansas, and Texas. Diseased plants exhibit a chlorosis of the terminal leaf parts, and panicles produce little grain. Prior reports attributing white tip to an ectoparasitic, seed-borne nematode, *Aphelenchoides besseyi* Christie, 1942 (syn. *A. oryzae* Yokoo, 1948), were confirmed. Histological studies demonstrated that the nematode was ectoparasitic. In infested seed stored at room temperatures, the nematodes remained viable for 23 months. Infested seed, not soil, was a source of inoculum. To grow the nematodes in artificial culture, nematode-infested rice seed were introduced into flasks containing a substrate of steamed, unhulled rice. Fungi from the partially disinfested seed, generally species of *Helminthosporium*, *Curvularia*, *Alternaria*, or *Fusarium*, growing on the moist substrate within the flasks served as a source of food for growth and multiplication of the nematodes. Since, in several attempts in various media, the nematodes could not be cultured in the absence of fungi, inoculations with pure cultures of the fungus components of the nematode cultures were tested, but were incapable of producing symptoms of white tip. Suspensions of nematodes from laboratory cultures consistently produced white tip symptoms in susceptible rice varieties.

For many years white tip has been an important panicle and leaf disease of rice in Arkansas, Louisiana, and Texas. Losses have been reduced in recent years by wider use of resistant varieties and control measures based on better knowledge of the disease. White tip is caused by an ectoparasitic seed-borne nematode, *Aphelenchoides besseyi* Christie. Before 1948 white tip was considered a physiological disorder.

Studies on white tip started in 1949 are included in the present paper. Abstracts have been published on certain phases of the work (13, 14).

REVIEW OF LITERATURE.—In the United States, Jodon (7) in 1935 was apparently the first to mention the rice disease known as white tip. Tullis and Cralley (16) reported on a chlorosis of rice caused by an iron deficiency. Though the disorder was called chlorosis, the symptoms described were those of white tip. Jones et al (8) and Tullis (15) attributed white tip to an iron deficiency, but Ryker (12) was unable to confirm such reports. Martin (9) and Martin and Altstatt (10) described the symptoms for white tip, studied the effects of magnesium and calcium, and concluded that the disease was due to magnesium deficiency. Thus, Tullis (15), Ryker (12), and other U. S. pathologists considered white tip a physiological disease of rice until 1949 (4), because the disease could not be attributed to any specific pathogen.

In 1950 Yoshii and Yamamoto (19) reviewed the early work on white tip in Japan. Yoshii, in 1943, was unable to confirm the work of Martin (9) attributing white tip to a magnesium deficiency. In 1944, finding that nematodes were associated with diseased plants, Yoshii named the disease Senchû Shingare

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TABLE 1.—*Comparison of panicles from normal and white-tip tillers*

	White-tip affected	Normal
Panicle length, average	15 cm	21 cm
Panicle length, range	10–22 cm	18–22 cm
Panicle weight, average	0.7 g	2.5 g
Panicle weight, range	0.3–2.2 g	1.3–3.4 g
No. grains per panicle, average	32	98
No. grains per panicle, range	8–87	48–131
No. sterile florets, average	19	19
No. sterile florets, range	6–44	6–57
Per cent sterile florets	37	16

Byô, "heart blight caused by a nematode." In 1946 Yoshii (18) reported the effects of hot-water seed-treatment tests. In the United States, Cralley (4) was the first to confirm the findings of Yoshii (18) and Yokoo (17) as to the nematode nature of white tip.

Yoshii (18), in 1946, used *Aphelenchoides oryzae* Yokoo, 1946, as the name for the nematode, and Yokoo (17) described the species in 1948. In a taxonomic study, Allen (1) considered *A. oryzae* Yokoo, 1948, to be a synonym of *A. besseyi* Christie, 1942. The writers accept the opinion of Allen as to the proper species name for the nematode.[4]

Yoshii and Yamamoto (21) concluded that the nematodes are seed-borne, and that the soil rarely, if ever, serves as a source of inoculum. They (20) also found that the nematodes could persist in stored rice for 3 years.

SYMPTOMS.—On susceptible rice varieties, the tips or terminal portions of the leaves become white or chlorotic for a distance up to about 5 cm, and these areas later are often dark or necrotic and frayed. The common name of "white tip" is based on the initial characteristic symptom (Fig. 1C). Frequently, the basal or middle parts of the leaves also show chlorotic areas. Diseased plants are reduced in vigor and height, and produce small panicles. Both the panicle length and the number of spikelets are reduced. The reduction is most evident in the terminal portion of the panicle, where lemmas and paleas often are absent. Affected panicles show excessive sterility, and the fertile florets at maturity have twisted or distorted glumes (Fig. 1B) and small, distorted kernels. Panicles on diseased tillers vary from essentially normal to nearly completely sterile.

In the field, severely diseased plants occur along with those showing milder or the more typical symptoms. The upper leaves, particularly the flag leaf of severely diseased plants, are markedly twisted. The panicles of such plants are often so tightly bound by the twisting of the flag leaf and subtending sheath that emergence from the boot is incomplete (Fig. 1A). The panicles of severely diseased tillers mature late, and culms with sterile panicles tend to remain in a vegetative condition and produce new growth before normal or less severely diseased plants reach maturity. Frequently, a single tiller shows a terminal, sterile panicle, with 1 or 2 later panicles that arise from the lower nodes.

To obtain information on the effect of white tip on panicle development, panicles from normal and severely diseased Zenith plants were tagged shortly after emergence. The plants were growing in pots of soil in an outdoor screened cage. The healthy plants were grown from hot-water-treated seed. At maturity the panicles were collected and compared. As shown in Table 1, white tip reduced panicle length and weight, and seed per panicle, and increased sterility.

STUDY OF THE PATHOGEN AND EXAMINATION OF DISEASED PLANTS.—After 1949 the reported (4, 17, 18) presence of nematodes in the seed was verified by microscopic examination of rice seed of susceptible varieties from fields and plots exhibiting foliar symptoms of white tip. In the laboratory, several seeds were dehulled by hand with forceps and placed in Syracuse watch glasses or small (50-mm) Petri dishes, together with the hulls, in a small quantity of warm water. The nematodes in a state of anabiosis gradually became active, as observed with a steroscopic microscope. A small percentage of the nematodes became active within an hour, but the maximum number in an active condition could be detected after 12–24 hours. The age of the seed, which is the length of time that the nematodes have been in a state of anabiosis, as well as the temperature of the water, influences the length of time for the nematodes to become active. Most of the nematodes were found on the inner surface of the hulls, but some occur on the kernel.

A limited study was conducted to determine the number of nematodes per seed using panicles of Early Prolific collected from white-tip plants at the Rice Experiment Station, Crowley, Louisiana. The number of viable nematodes per seed was 1–14, averaging 6.6.

Infected rice plants in the late tillering and boot stages were collected from the field. The older, or lower, leaves were stripped off and the younger, unfolded, or meristematic, leaves and floral parts were cut into small pieces and placed in water in Syracuse watch glasses. Rather large numbers of nematodes were observed in the water.

A study was made to determine whether or not the nematodes entered the plant tissues. The staining techniques used were those described by Godfrey (5), Goodey (6), and McBeth, Taylor, and Smith (11). Root, culm, leaf, and panicle portions from plants showing severe white tip symptoms were thoroughly washed, hand-sectioned, stained, and examined microscopically. No nematodes were observed within stained tissues of the various plant parts. However,

[4] The writers acknowledge the assistance of A. L. Taylor, Nematology Section, Agricultural Research Service, U. S. Department of Agriculture, in identifying nematode specimens.

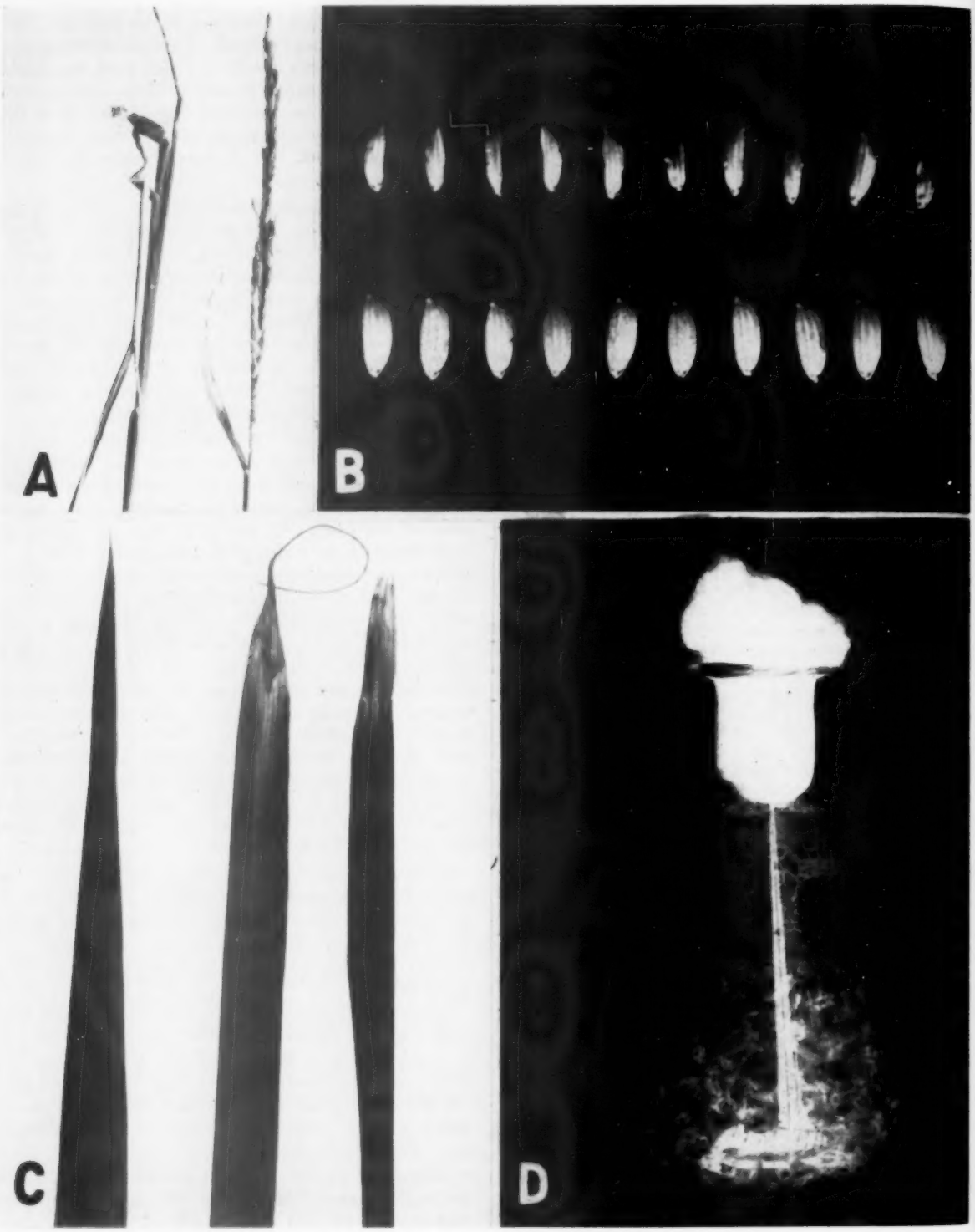

FIG. 1. A) Effect of white tip on panicles. On left, extreme symptoms. B) Effect of white tip on seed, Zenith Variety. Bottom row, normal seed. C) Typical leaf symptoms of white tip. Normal leaf on left. D) Laboratory culture of *Aphelenchoides besseyi*.

nematodes were recovered from leaf sheath (boot) washings. These studies confirmed the ectoparasitic nature of this foliar nematode.

LONGEVITY OF THE NEMATODE IN RICE SEED.—The longevity of the nematodes in a dormant state in rice seed was determined in a periodic examination of stored samples—by hulling and washing the seed and hulls. The samples used were collected in September, 1949, at the Rice Experiment Station, Crowley, Louisiana, and kept in manila envelopes at room temperatures of 70–90°F.

Some of the nematodes remained viable in dry rice seed up to 23 months, but not for 24 months. The rice seed also was essentially nonviable at the end of 24 months of such storage. The nematodes decreased in viability and vigor with time, from the initial examination, shortly after collection of the samples. The study indicated that prolonged storage was not a practical method of securing "disease-free" seed rice, because of the concomitant loss in germination. The moisture content of the seed was not determined, but was probably about 12%.

LABORATORY CULTURE OF A. BESSEYI.—Attempts were made to rear the nematodes in the laboratory in artificial culture, because of the need for large numbers for pathogenicity tests. Preliminary attempts, using media and methods similar to those used for the artificial culture of fungi and bacteria, were not successful.

The nematodes were successfully cultured by the following procedure: Erlenmeyer flasks partially filled with unhulled rice, with sufficient water added, were plugged with cotton and sterilized in an autoclave. The nematode-infested seeds were first immersed in 1:1000 mercuric chloride for 3–5 minutes and then washed with a 1:5 solution of Clorox (sodium hypochlorite). This surface disinfestation reduced contamination by species of *Aspergillus* and *Penicillium*, and by certain Mucorales that apparently were on or in the hulls, but did not drastically affect the nematodes. About 10 of the surface-sterilized nematode-infested rice seed were dropped into each flask with sterilized forceps. Following inoculation, the flasks of culture media were held at room temperatures of 70–90°F or in a constant-temperature room at 70°F. The rice seed added to the flasks germinated, but the seedlings soon died. Fungi not eliminated by the surface-disinfestation treatment grew from the rice seed and developed rapidly on the culture medium. After about 6 weeks, large numbers of nematodes were observed along the walls of the culture flasks. These had a characteristic lace-like appearance, as shown in Fig. 1D. The cultures, as illustrated, contained thousands of nematodes. Erlenmeyer flasks used were of capacities 250–2000 ml. Subcultures were utilized sometimes. All of the culture flasks containing large numbers of nematodes contained fungi, generally species of *Alternaria*, *Curvularia*, *Helminthosporium*, or *Fusarium*. Those overrun by species of *Aspergillus* or *Penicillium* or by the Mucorales contained fewer nematodes, but the nematodes failed to multiply in flasks containing no fungi. Bacteria were also present.

After this method had been developed for laboratory culture of *A. besseyi*, the writers noted that the same basic method had been used previously—by Christie and Crossman (3) for summer dwarf nematode (*A. besseyi*), and by Christie and Arndt (2) for other nematodes in the same genus.

Results were negative in attempts to secure multiplication of the nematodes from flask cultures in the absence of fungi. For these studies, shredded blood fibrin or blood fibrin extract was added to corn-meal agar plates as a protein source in various combinations, with or without low concentrations of lactic acid or malachite green. Nematodes from flask cultures were washed in sterile water or 1:2000 mercuric chloride (followed by 2 changes of sterile water) and concentrated by centrifuging. The volume of the nematode suspension was adjusted so that each ml contained about 10 nematodes. Following the addition of 1 ml to each plate of culture medium, they were held 30 days at 30°C and examined periodically under a microscope. The nematodes failed to multiply in culture dishes that were not contaminated with fungi, but those containing fungi permitted multiplication. It was concluded that fungi were necessary for growth and multiplication of the nematodes.

PATHOGENICITY STUDIES.—In a preliminary test, a sample of Early Prolific rice seed from a lot known to be infested with nematodes was treated 15 minutes with hot water at 54°C. Treated and untreated rice was seeded in 5-gal. glazed jars of soil from the Rice Experiment Station. Before seeding, the soil in half of the jars was treated with chloropicrin and then aerated. The plants were observed periodically and rated for white tip until after heading.

The hot-water treatment eliminated white tip except for 1 pot of the series treated with chloropicrin. The occurrence of white tip in the 1 pot possibly resulted from contamination from other pots or incomplete elimination of nematodes from the seed, since a presoak treatment was not used. Treatment of the soil with chloropicrin had little or no effect on the disease. This experiment showed that white tip was caused by a seed-borne pathogen that could be controlled by a hot-water treatment. Similar results were obtained in a later test, with soil from 2 farms where white tip had been observed. However, white tip occurred in a series of jars used for pathogenicity tests the previous season.

The effect of time of inoculation and source of inoculum was studied in another pathogenicity test. In this experiment, nematodes from laboratory cultures and infested seed were used as inoculum. The rice was treated 15 minutes with hot water at about 54°C and seeded in 2-gal. jars filled with steamed soil. In certain of the pots, macerated infested grains were mixed into the soil surface at seeding. After emergence the plants were thinned to 6 per pot. Plant inoculations were made when the rice plants were

TABLE 2.—*White tip incidence in an experiment using 2 sources of inoculum and different inoculation techniques*

Inoculum source	Method of inoculation	Stage of growth	White tip per cent [a]
Control (water)	Syringe	12–15 in.	0
Control (water)	Syringe	Late boot	0
Infested grain	Soil	Seeding	83
Laboratory culture	Soil	Seeding	42
Laboratory culture	Syringe	12–15 in.	100
Laboratory culture	Syringe	Late boot	70

[a] Average of 4 replications.

12–15 in. tall. Syringe inoculation of plants consisted of introducing the nematode suspension under the leaf sheath.

The results (Table 2) showed that nematodes from laboratory cultures were as capable of inducing white tip as were those from infested seed. Inoculation of young plants (12–15 in. high) with a suspension of nematodes applied with a syringe gave 100% infection, and this method was adopted for further use.

Pure cultures of bacteria and fungi from flask cultures of nematodes were isolated, and young rice plants up to 15 in. tall were inoculated with these cultures by the syringe method. None of these inoculations induced white tip.

Laboratory cultures of nematodes were used for field inoculation in replicated 2×2-ft. plots at the Rice Experiment Station in 1951 in an area that had not been planted to rice for the 5 previous years. Galvanized-iron forms served as a barrier against movement of the nematodes. Laboratory cultures were mixed into the upper soil surface to provide heavy infestation. The plots were inoculated and seeded immediately, on June 21, 1951. Seed of 5 rice varieties was treated with hot water to eliminate seed-borne nematodes, and dried before sowing. The control plots received no inoculum. The soil within the metal forms was kept moist by frequent watering until the rice was several inches tall. After the field was flooded, however, seepage was sufficient to maintain the water within the metal forms at a shallow depth. Notes as to the presence of white tip were taken at the boot stage of growth. At maturity the plots were harvested, and the seed was examined for nematodes. Data are given in Table 3.

TABLE 3.—*Field test of laboratory cultures of nematodes for infesting soil at seeding*

Variety	Treatment	White tip	Nematodes per sample [a]	Yield: Grams per plot [b]	Yield: Decrease (%)
Early prolific	Soil infested	+	12	148	43.3
Early prolific	Control	—	0	261	
Blue Rose	Soil infested	+	13	149	40.2
Blue Rose	Control	—	0	249	
Zenith	Soil infested	+	17	184	26.1
Zenith	Control	—	0	249	
Arkrose	Soil infested	+	17	201	18.6
Arkrose	Control	+	17	247	
Bluebonnet	Soil infested	—	1	338	7.1
Bluebonnet	Control	—	0	364	

[a] Ten seed per sample.
[b] Averages for 3 control plots and 5 infested plots.

White tip was produced, and nematodes were recovered, from the grains in the inoculated plots of Arkrose, Blue Rose, Early Prolific, and Zenith. The resistant Bluebonnet variety showed no white tip. Control plants of Arkrose also showed white tip and nematode occurrence, probably as a result of incomplete seed treatment. The results of the test suggested that the method could be used on a somewhat larger scale for field-testing rice varieties and selections for resistance to white tip.

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ANATOMY OF FUSIFORM RUST GALLS ON LOBLOLLY PINE[1]

L. W. R. Jackson and J. Neil Parker[2]

SUMMARY

The fusiform rust fungus causes the development of perennial spindle-shaped galls on branch or stem of loblolly and slash pine. Hyphae are large, septate, and uninucleate. The haustoria, which become greatly constricted as they pass through the cell wall, are allantoid, unbranched, and uninucleate. In young twigs, the hyphae freely ramify through the intercellular spaces of the loosely arranged parenchyma cells in the primary bark tissue known as cortex. In the older galls, they advance radially along the phloem rays through the cambium and the wood rays to a depth of 2–3 rings. The haustoria invade parenchymatous cells in the cortex, phloem, and wood rays, and cells in the resin ducts. The hyphae do not invade the tracheids. Infection greatly increases the size and number of the cells in the wood rays.

During the past 2 decades, the incidence of fusiform gall rust caused by *Cronartium fusiforme* Hedgc. & Hunt. has greatly increased on slash pine (*Pinus elliottii* Engelm.) and loblolly pine (*P. taeda* L.) in the pine forests of the southern United States. The fungus either kills the tree, by girdling the stem, or weakens it to the point that wind pressure breaks it. Many investigators (3, 6, 7, 8, 9) have reported on the life history of the rust disease and the site factors that affect its incidence. This study was primarily concerned with the pathological anatomy of the rust galls on loblolly pine.

TECHNIQUES.—Throughout the winter season, 2- and 3-year-old branch galls were taken from 7-year-old planted loblolly pines. The specimens were fixed in Jackson's FAA solution (5). Sections were cut on either the sliding microtome or with the freezing attachment. Of the several stains used to differentiate fungus mycelium in the gall tissues, Cartwright's safranin and picro-anilin blue (1) gave the best results. Safranin and fast green gave good differentiation of the various tissues. All sections were permanently mounted in Diaphane.

Fungus component.—The fusiform rust fungus could be distinguished from other fungi found in pine infections by the relatively large and uniform diameter of the hyphae and the haustoria in the parenchymatous cells. The diameter of the intercellular hyphae (Fig. 1-AB) in the wood rays varied from 3.0 to 4.3 μ, averaging 3.5. Colley (2) gave the diameter of white pine blister rust (*Cronartium ribicola* Fischer) as 3.0–3.5 μ. According to Hutchinson (4), the diameter of the hyphae of the sweetfern rust (*Cronartium comptoniae* Arthur) varied from 3.6 to 6.2 μ. The hyphae were septate, and each cell contained a single small nucleus. There was considerable variation in the length of hyphal cells. In the cortex, where branches and haustoria were frequent, the hyphal cells were short. The longest cells occurred in the hyphae, along the phloem and wood rays.

The fusiform rust fungus, like others described (2, 4) was found to be intercellular throughout the gall tissues. In young twigs the hyphae freely ramify in the intercellular spaces of the loosely arranged parenchyma cells in the primary bark tissue, commonly called the cortex. In older galls they advance radially along the phloem rays through the cambium, in a right-angle direction, and then follow the wood rays. The hyphae (Fig. 1-AB) occur in the angle formed by the horizontal and vertical walls of the ray cells. Thus, a single ray cell may have a hypha on each of its 4 radial margins. In this manner, a single hypha can penetrate the cells above and below it. Nearly all ray cells contain at least 1 haustorium, whereas a few had 2, 3, or even 4. There was no tangential communication between rays except where a resin duct was confluent with 2 of them.

The hyphae occasionally extended from the horizontal boundaries of the rays into the angles between the tracheids of the xylem, in an apparent attempt to

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reach other rays. They bore numerous short branches, which entered the tracheid through the bordered pits. Growth of these hyphae terminated in the intervening areas of wood. It appeared that, unless the hyphae reached other rays, they could not be supported, and consequently died. The rust fungus did not occur in

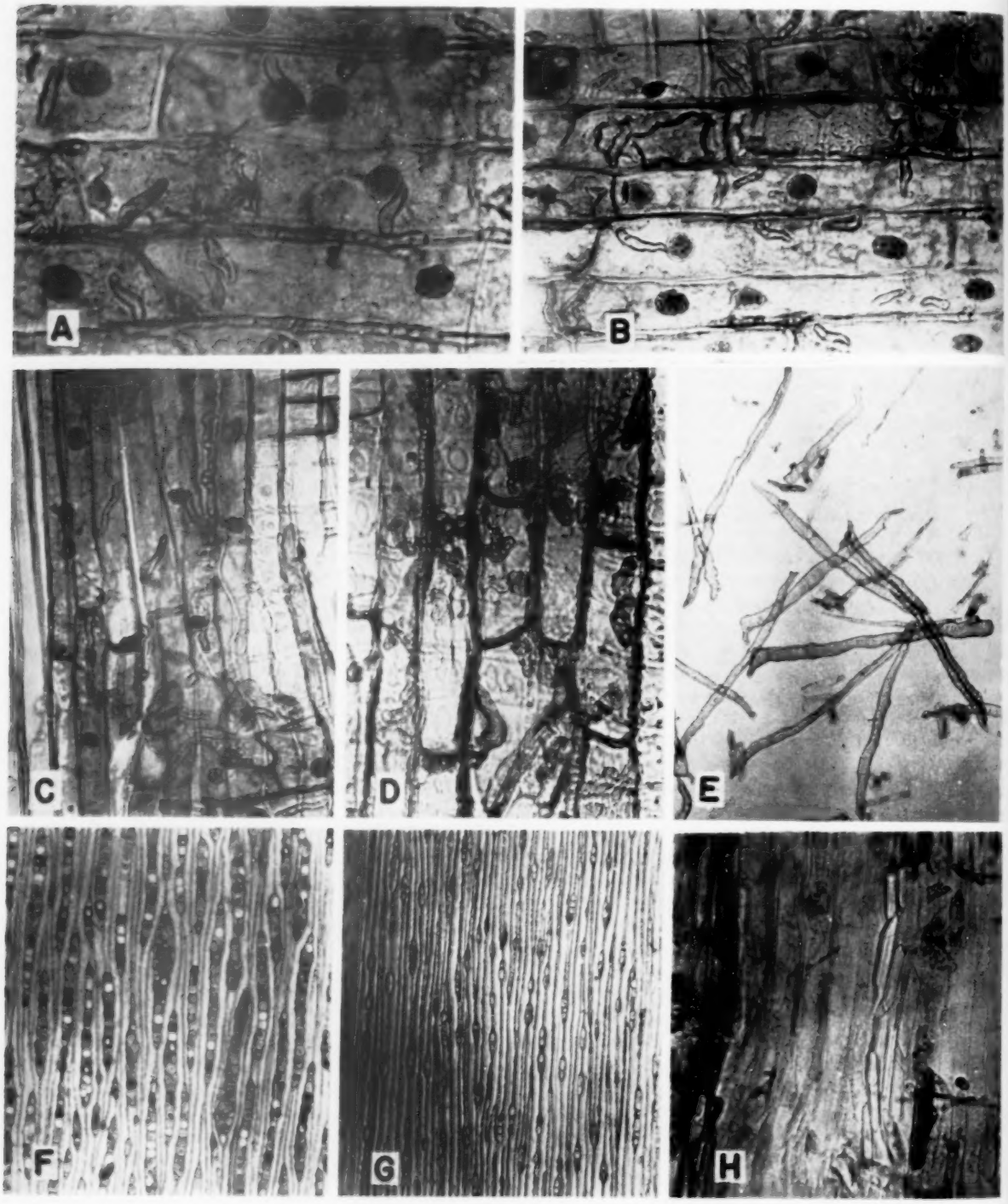

FIG. 1. A–B) intercellular hyphae and haustoria in wood rays (respectively ×900 and ×400). C) Haustoria in epithelial cells of a resin duct (×400). D) Dead haustoria coated with oxidation products (×900). E) Distorted tracheids from gall wood (×50). F) Enlarged wood rays in a gall (×100). G) Wood rays in healthy wood (×100). H) Crystals in gall phloem (×400).

the branch wood or bark beyond the externally visible margins of the gall.

In the resin ducts, because of the orderly arrangement of the epithelial cells, the mycelial pattern was very regular. The hyphae (Fig. 1-C) were in the angles formed by the cell walls, from which haustoria methodically penetrated the adjacent cells. The haustoria freely invaded the parenchymatous cells in the cortex, phloem rays, wood rays, and resin ducts. No mycelium was observed in the pith. Haustoria (Fig. 1-ABC) developed from branches of intercellular hyphae that became constricted to about 1.0 μ in width as they passed through the cell walls. Within the cells they expanded to the regular size of the hyphae. The unicellular haustoria were allantoid, uninucleate, unbranched, and 20–30 μ long. Many of the long ones were slightly twisted or contorted. Colley (2), Hutchinson (4), and True (10) reported that the host nuclei appeared to attract the haustoria. There was no evidence that the host nuclei attracted the haustoria of the fusiform rust fungus. Well-developed haustoria were observed in a few of the wood ray cell initials undergoing the last stages of mitosis, which indicated a very delicately balanced host-parasite relationship.

Host component.—The fusiform rust fungus causes the growth of long spindle-shaped swellings on the stem and branches of loblolly pine and slash pine. No summerwood forms in the annual rings of the gall. In the 2-year-old rings the tracheids (Fig. 1-E) were very irregular in shape and only 1.14 mm long, compared to 1.72 mm in the adjoining healthy branch wood.

Growth of the wood rays was modified even more than that of the tracheids. In healthy wood (Fig. 1-G) the height of the uniseriate rays varied from 1 to 5 cells. Average height of the gall rays (Fig. 1-F) was 10 cells, whereas some of the rays were 30–50 cells in height. A single mm^2 had 60 rays on the tangential sections of healthy wood, compared to 45 in the gall wood. But the healthy wood had only 140 ray cells per mm^2 compared to 550 in the gall wood. Many of the gall wood rays increased so much in height that they became confluent with upper and lower rays. An increase in the number of ray cells was accompanied by an increase in cell size. The mean radial length of ray cells increased from 73 μ in healthy wood to 103 μ in gall wood. True (10) found that the Woodgate Peridermium on Scotch pine (*Pinus sylvestris* L.) also caused an increase in the number, height, and cell volume of the rays in both phloem and xylem.

The rust fungus infections also invoked an increase in the number of vertical resin ducts in the wood. On transverse sections, there were 262 ducts per cm^2 in healthy wood, compared to 458 in gall wood. The ducts often occurred in tangential couplets or triplets. The number and size of the cells surrounding the canals were also increased.

With the growth of the gall, all the tissues in the bark increased in size. Secondary phloem was produced at a rate higher than normal, and the individual cells were larger. The phloem rays underwent a transformation similar to that in the wood rays. In the phloem, there were numerous cells of small diameter, bearing abundant styloid crystals (Fig. 1-H). As the gall aged, the phloem ray cells continued to swell, until they became isodiametric and lost their identity. The sieve tubes and crystal-bearing cells became obliterated by crushing. This trend reached its ultimate development prior to spore formation, when the parenchyma cells became dissociated and appeared as spherical bodies in a matrix of fungal mycelium.

Within the mature phloem there were numerous radial resin ducts that originated in the fusiform rays. As the rays lost their character the canals greatly increased in diameter, through repeated division of the epithelial cells.

Starch grains were usually abundant in newly formed parenchyma cells in and around the infections in galls collected during the winter season. As the host cells and the haustoria aged, the starch gradually disappeared. This was followed by an increase in the amount of tannin-like substances. Cells that contained granular deposits and fatty globules were abundant in the gall tissues and ray cells. Following the degeneration and death of the wood ray cells, the cell walls and haustoria (Fig. 1-D) became coated with a heavy deposit, probably a mixed oxidation product of the cell contents, fats, resins, and tannins.

The mycelium of the rust fungus remained dormant throughout the winter. Both the intercellular hyphae and host ceased growth and resumed activity in the spring at about the same time.

In the healthy part of the 2-year-old branch, the initial phellogen had already formed, but the epidermis remained intact. In the gall, the epidermis had largely disappeared and a second phellogen had formed below the cortex. In a few instances a third phellogen layer was found already cutting out small plates of secondary phloem. True (10) illustrated an example of an infection by the Woodgate rust fungus that had been completely walled off in the early growth stage. Although this type of host reaction occurred in fusiform rust galls, observations showed that, once the hyphae reached the phloem, the periderm barriers could do no more than retard the progress of the fungus.

Two host reactions to the presence of the rust fungus fall within the sphere of hormonal influence: the production of periderm barriers immediately in front of the advancing hyphae in the phloem, and the differential break of dormancy. Specimens collected in March were still dormant. In transverse sections of galls that partially girdled the branch, the first new tracheids had formed slightly in front of and in the vicinity of the leading hyphae. Newly formed tracheids could not be found in the uninfected part of the branch or in tissues where the hyphae were

well established. In the vicinity of the advancing hyphae, the formation of some kind of growth-promoting substance apparently stimulated an early break of dormancy.

Older cankers frequently bore numerous holes, made by birds seeking larvae of a chalcid wasp of the family Eurytomideae, tentatively assigned to the genus Bephratoides. The larvae of this wasp did not excavate galleries at the interphase of the phloem and xylem, but occupied radially placed chambers in the xylem that barely touched the inner edge of the phloem. Most of the galls had at least 2 larvae, but some medium-sized galls had as many as 20–30. The adults emerged from caged specimens during the last week in February, and within 2 days were probing the bark of the gall with their ovipositers in an attempt to lay eggs. It is believed that the concentration of food-rich tissues and the presence of the mycelium in the rust gall afforded an ideal habitat for the breeding of the chalcid wasp.

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A PHYTOPATHOLOGICAL NOTE

Acti-dione as a Control of Seed-borne Safflower Rust. EUGENE M. WILSON AND PETER A. ARK.[1] Eight per cent of a collection of safflower (*Carthamus tinctorius* L.) seed from Oregon produced seedlings infected with safflower rust, *Puccinia carthami* Cda. To determine the efficacy of aqueous solutions of Acti-dione (β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide) in controlling seedling infection by this rust, seed from this collection was soaked 30 minutes in 1, 3, 5, 10, 15, 20, 100, or 1,000 ppm Acti-dione solutions. After drying 3 hours at room temperature, the seeds were planted in flats of sterile soil in the greenhouse. Each treatment consisted of 300 seeds; all treatments were repeated at a different time. The appearance of 1 or more pustules on a plant by 21 days after planting was the criterion for infection. Appropriate untreated and water-treated checks were included.

Complete control of seed-borne rust was obtained at all concentrations tested. At 1, 3, 5, 10, 15, and 20 ppm there was no apparent reduction in per cent germination or size of seedling; at 100 ppm, germination was reduced 5–6%; at 1,000 ppm, 82%. At these last 2 concentrations seedling emergence lagged 3–10 days.

In a similar study on the seed-borne stage of this disease, Schuster[2] reported that seed treatment with Acti-dione at 250 or 1,000 ppm decreased emergence of safflower seedlings, but that emerged seedlings were free of visible rust infection. The present study corroborates his observations and suggests the use of low concentrations of Acti-dione as an effective control of the seed-borne stage of this disease.—Department of Plant Pathology, University of California, Berkeley, California.

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[2] Schuster, M. L. 1956. Investigations on the foot and root phase of safflower rust. Phytopathology 46: 591-595.